Electrochemical Investigation and Analytical Determination of Nucleic Acid Constituents at Different Carbon Composite Electrode Surfaces

By

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Dhahran, Saudi Arabia

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DEDICATED

To My

Parents, Brothers and Sisters

ACKNOWLEDGMENT

I would like to begin by thanking Allah, Lord of the worlds, for everything. I am asking him always to forgive me and direct me to the right road.

I would like to express my deep thanks to King Fahd University of Petroleum & Mineral for the support and for giving me the chance to study and achieve what I have achieved.

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THESIS ABSTRACT

Name: Abdulnaser Khaled Alsharaa

Title: Electrochemical Investigation and Analytical Determination of Nucleic acid constituents at Different Carbon Composite Electrode Surfaces

Major Field: Chemistry

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The attractive stripping performance of nucleic acid constituents were characterized at different carbon composite electrode surfaces. A simple, sensitive and reliable electrochemical method was developed for the investigation and analytical determination of adenine (A), guanine (G), adenosine (AS) and guanosine (GS) at different composite carbon electrode surfaces. Different electroanalytical techniques were used as cyclic voltammetry (CV), square wave stripping voltammetry (SWV), linear sweep voltammetry (LSV) and differential pulse voltammetry (DPV). Factors influencing the trace detection of these compounds at glassy carbon paste (GCP), glassy carbon (GC) and graphite pencil (GP) composite electrode surfaces were assessed.

All developed methods were optimized and the figures of merit were determined. The possibility of the detection in biological samples, interference of common organic compounds, and direct determination of ssDNA were tested successfully. Finally the developed methods were compared to HPLC.

Master of Science Degree

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ملخص الرسالة

الاســــــــــم : عبد الناصر خالد الشرع

عنوان الرسالة : الفحص الكهركيميائي والتحديد التحليلي لمكونات الحمض النووي على أسطح أقطاب الكربون المختلفة

التخصـــــص : كيمياء

تاريخ التخـرج : يونيه 2010

لقد تمت دراسة السلوك الكشطي الكهروكيماوي لمكونات الحمض النووي على أسطح أقطاب الكربون المختلفة. وتم تطويرطريقة كهركيميائية بسيطة وحساسة وموثوقة من أجل الفحص والتحليل للأدينين والجوانين و الادينوزين و الجوانوزين على أسطح هذه الاقطاب الكربونية الجديدة. ومن التقنيات الكهركيميائية المستخدمة في الدراسة الفولتامترية الدائرية (Cyclic Voltammetry) والفولتامترية الكشطية رباعية الموجة (Square-Wave Voltammetry) والفولتامترية المسحية الخطية (Linear-Sweep Voltammetry) والفولتامترية الكشطية النابضة (Differential Pulse Voltammetry).

وقد تمت دراسة مختلف العوامل المؤثرة على تقدير تركيزات دقيقة من هذه المركبات محل الدراسة على أسطح الاقطاب الكربونية الكهربائية التالية: عجينة الكربون الزجاجي (Glassy Carbon Paste) وكذلك الكربون الزجاجي(Glassy Carbon) والكربون الجرافيتي (Graphite Pencil).

لقد تمت أيضا دراسة وتحديد أفضل الشروط المناسبة لهذه الطرق المطورة. وكذلك امكانية استخدام هذه الطرق لتحديد هذه المكونات في وسط بيولوجي وكذلك دراسة تأثير وجود بعض المركبات العضوية الشائعة. وقد أثبتت الطرق المطوّرة نجاحها للتحديد المباشر للحمض النووي (ssDNA). وأخيراً تمت بنجاح مقارنة هذه الطرق الكهربية المطوّرة مع تقنية الكروماتوجرافيا السائلة عالية الأداء (HPLC).

درجة الماجستير في العلوم

جامعة الملك فهد للبترول والمعادن

الظهران-السعودية

CHAPTER 1

1. Introduction and Literature Review

1.1. Electroanalytical Techniques:

Electroanalytical techniques are concerned with the interplay between electricity and chemistry, namely the measurements of electrical quantities, such as current, potential, or charge, and their relationship to chemical parameters. Such use of electrical measurements for analytical purposes has found a vast range of applications, including environmental measurements (1), industrial quality control (2), and biomedical analysis (3). They have many advantages such as high sensitivity, good selectivity, rapid response, low cost and simplicity.

One of the most useful branches of electrochemistry is voltammetry. The analytical advantages of the various voltammetric techniques include excellent sensitivity with a very large useful linear concentration range for both inorganic and organic species, a large number of useful solvents and electrolytes, a wide range of temperatures, rapid analysis times (seconds), simultaneous determination of several analytes, the ability to determine kinetic and mechanistic parameters, a well-developed theory and thus the ability to reasonably estimate the values of unknown parameters, and the ease with which different potential waveforms can be generated and small currents measured.

1.1.1. Cyclic Voltammetry:

Cyclic voltammetry is mainly used for qualitative analysis and usually performed in any electroanalytical studies. It gives clear information about kinetic and thermodynamic of redox processes, electron transfer reactions and adsorption processes. It rapidly provides location potentials of electroactive species (4).

Cyclic voltammetry consists of scanning linearly the potential of the working electrode using a triangular wave form, (Figure 1.1), and during the potential sweep the potentiostat measures the resulting current. Single or multiple cycles can be used depending on the information sought. Cyclic Voltammogram can be divided into three types, reversible, (Figure 1.2), quasi reversible, and irreversible. The characteristic peaks in the cyclic voltammogram are caused by the formation of the diffusion layer near the electrode surface (5).

1.1.2. Differential Pulse Voltammetry:

Differential pulse voltammetry is a useful technique used mainly for the analysis of trace levels of organic and inorganic compounds. In this technique pulses of fixed magnitude are applied to the working electrode, (Figure 1.3). The resulting plot of current vs. potential is termed as a differential pulse voltammogram. The height of the current peaks is proportional to the concentration of the corresponding analytes (6).

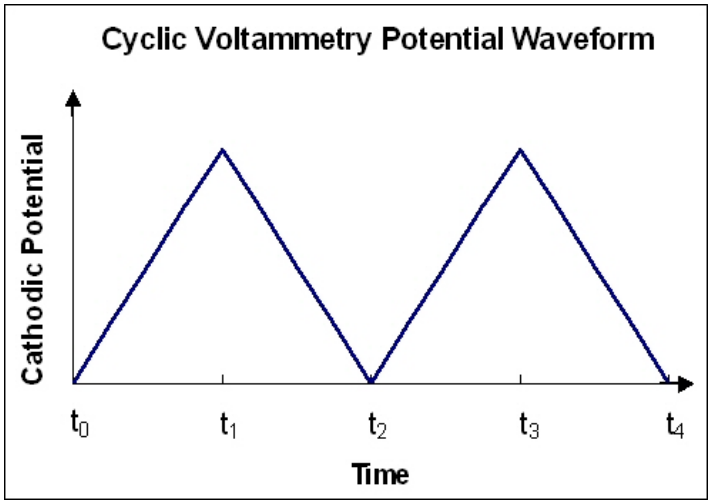


Figure 1.1. *Potential-time excitation signal in cyclic voltammetric experiment* (5).

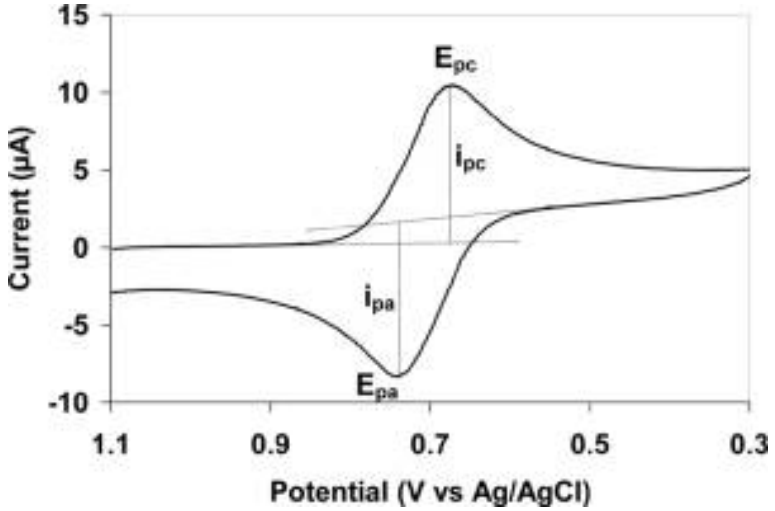


Figure 1.2. *Typical cyclic voltammogram, where i_{pc} and i_{pa} show the peak cathodic and anodic current respectively for a reversible reaction* (5).

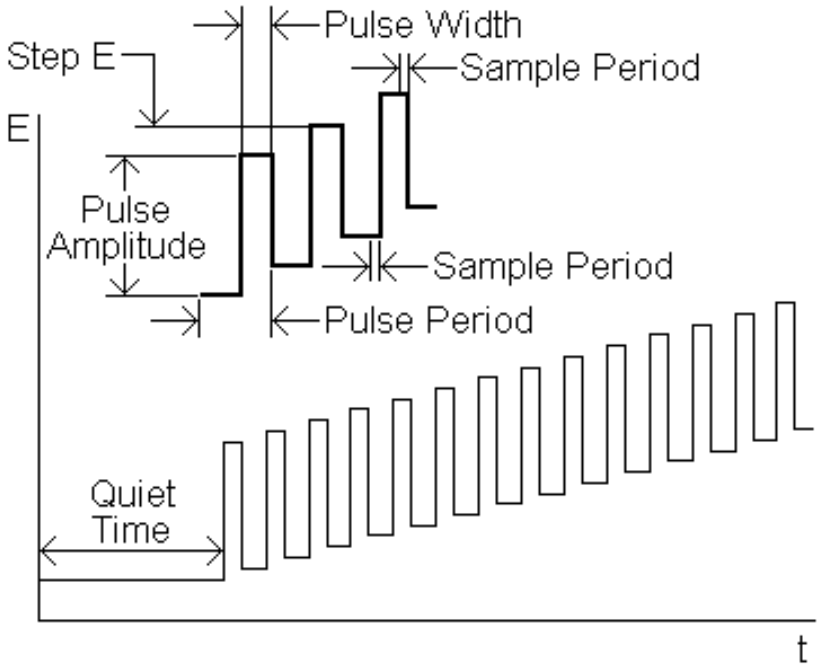


Figure 1.3. *Potential wave form for differential pulse voltammetry* (6).

1.1.3. Square Wave Voltammetry:

Square wave voltammetry is a pulse voltammetric technique in which a waveform composed of a symmetrical square wave is applied on the working electrode (7). The current is measured at the end of the forward pulse and at the end of the reverse pulse, (Figure 1.4).

Many advantages for square wave voltammetry such as high speed and very low detection limit near 1×10^{-8} M can be achieved. From the rapid scanning capability and the reversal nature of the square wave voltammetry, kinetic studies can be undertaken.

As a comparison between square wave and differential pulse voltammetry for reversible and irreversible cases, results indicate that the square wave currents are higher than the differential pulse response with 4 and 3.3 times respectively (8).

1.1.4. Linear Sweep Voltammetry:

Linear sweep voltammetry is a voltammetric method where the current at a working electrode is measured while the potential between the working electrode and a reference electrode is swept linearly in time. Oxidation or reduction of species is registered as a peak or trough in the current signal at the potential at which the species begins to be oxidized or reduced, (Figure 1.5) (4).

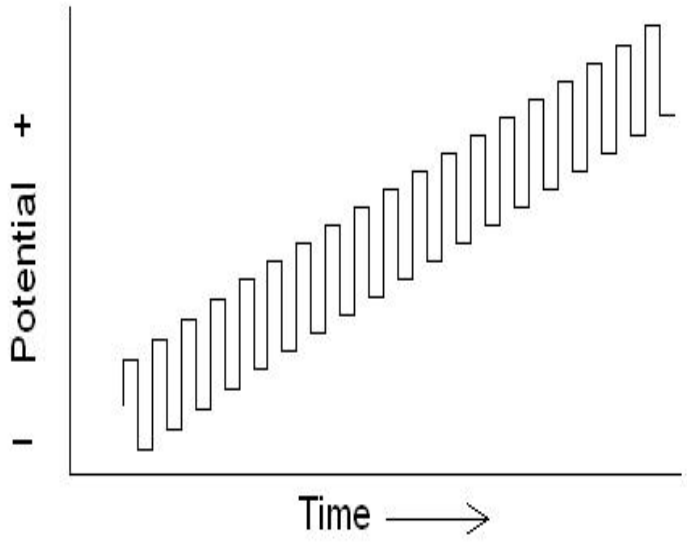


Figure 1.4. *Square wave potential sweep (4).*

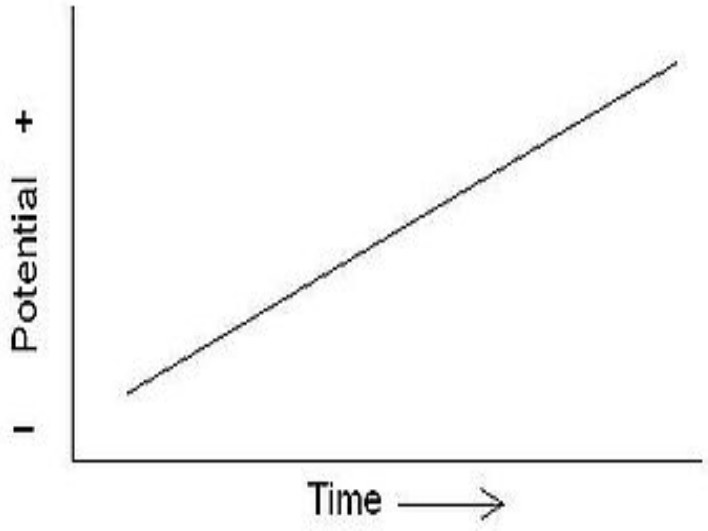


Figure 1.5. *Linear potential sweep* (4).

1.2. Nucleic Acid Constituents:

Deoxyribonucleic acid (DNA) is a straight, right handed double helix composed of two stands held together. It plays a crucial role in the storage of genetic information and in protein biosynthesis. The DNA structure, (Figure 1.6), is a copolymer of phosphoric acid and 2-deoxyribose, with one of the four bases, adenine (A), guanine (G), cytosine (C) and thymine (T), (Figure 1.7), linked to C-1 of each pentose unit.

1.2.1. The Chemical Structures and Properties of Nucleic Acid Constituents:

1.2.1.1. Bases:

The bases in DNA can be divided into two groups, purines which include adenine and guanine, and pyrimidines which have thymine and cytosine.

As shown in Figure 1.7, adenine contains an amino group ($-NH_2$) on the C6 position of the purine ring (carbon at position 6 of the purine ring), while guanine contains an amino group at the C2 position and a carbonyl group at the C6 position.

From the other hand, thymine has a hydrogen atom at the C6 position with carbonyl groups at the C2 and C4 positions. Cytosine has a hydrogen atom at the C5 position and an amino group at C4.

The bases have very important roles as the informational molecules in the cell. The purines and pyrimidines have a unique structure identity because the hydrogen bond donor and acceptor groups have different placement and that allows them to serve as the genetic information (9).

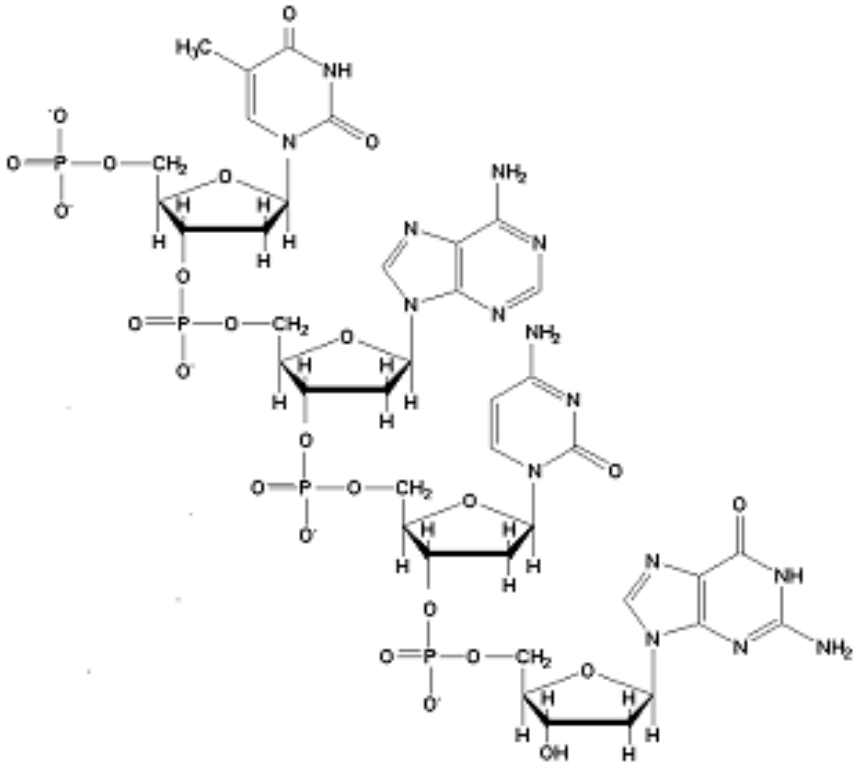
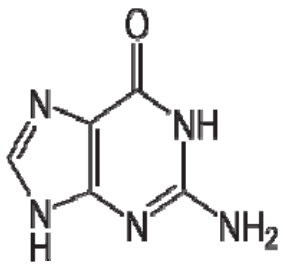
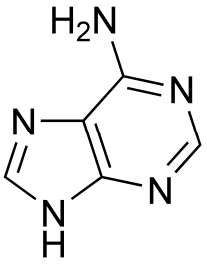


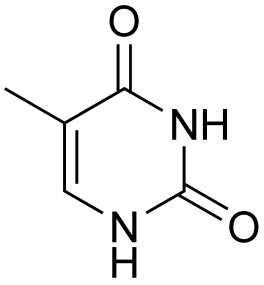
Figure 1.6. *The chemical structure of single-stranded DNA (10).*



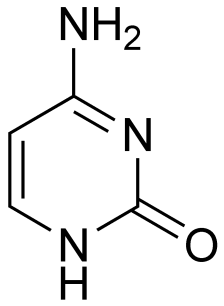
Guanine



Adenine



Thymine



Cytosine

Figure 1.7. *The chemical structures of nucleic acid bases (11).*

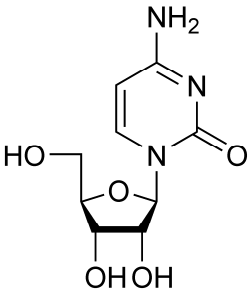
1.2.1.2. Nucleosides:

The term given to the combination of bases and sugar is nucleosides. Figure 1.8 shows the chemical structure of the four nucleosides related to the previous bases, adenosine, guanosine, thymidine and cytidine.

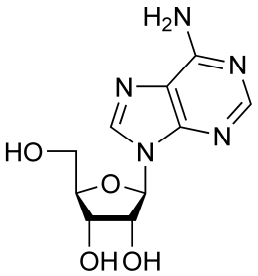
In DNA there are two types of sugar, ribose and deoxyribose, (Figure 1.9). The bond between the sugar and the base is called glycosidic bond. It is place in the β (up) configuration with respect ribose sugar, in contrast to the α (down) position of the hydrogen. The two standard configurations of the bases around this bond are syn and anti. These compounds are very well-known for their metabolic and biological effects in human system. Their detection and determination have become increasingly important in the field of biomedical research owing to their importance in normal cellular functions (12).

1.2.2. Chemical Properties of Nucleic Acid Constituents:

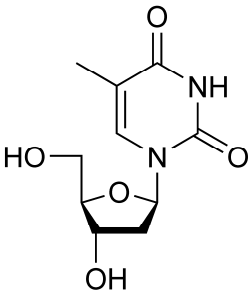
In 1960s, the electroactivity of nucleic acid constituents was discovered (13). V. Vetterl demonstrated the ability of nucleic acid bases to associate at the electrode surface in the late 1960s (14, 15). From the all constituents, only bases show a good redox reaction at different electrode surfaces, and sugar residues oxidize at copper electrode surfaces. Bases can be oxidized at carbon electrodes and undergo reduction at mercury electrodes (16). Adsorption phenomena takes place when the nucleic acids interact with electrodes, the signal produced due to the charge transfer reactions happen with the adsorbed nucleic acids that can give information about their types and concentrations.



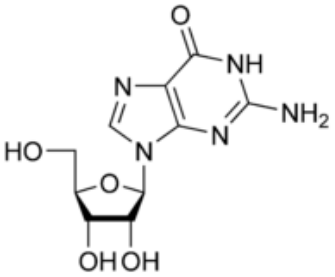
Cytidine



Adenosine

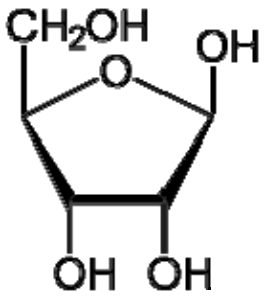


Thymidine

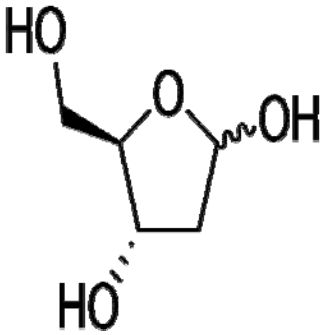


Guanosine

Figure 1.8. *The chemical stractures of nucleosides (9).*



D-Ribose



Deoxyribose

Figure 1.9. *The chemical structure of β-D-ribose, and β-D-deoxyribose.*

In addition, all bases and some of their derivatives have anodic signals due to the formation of sparingly soluble compounds with the mercury electrode (17, 18).

The reduction and oxidation process of nucleic acid constituents at electrodes are irreversible at high negative and positive potentials (19). With some interaction between DNA and electroactive intercalative drugs and other compounds, Berg et al. were able to obtian reversible signals (20, 21).

One of the important properties of DNA is the interaction with metal ions, because that plays a central role for the normal processing of DNA in the living cell (22). However, after exposure to a potential toxic metal agent lethal damage can be happened due to the kinetic competition between DNA interaction and binding other cellular components. Metal ions interact with nucleic acids and stabilize or destabilize the double helix (23). The nature of the metal, its size and charge affect the conformation and the structure of the product (24). The transition metal ions interact with N7 position of purines and N3 site of pyrimidines, by forming a chemical bond, M-N7 or M-N3 respectively (25).

According to literature, Kawde et al. have long been interested in the development of electrochemical sensors of different analytes (26-52). Out of these sensing protocols, there are few developed for sensing sugars (28, 29), aptamers (30, 32, 33), nucleic acids (31, 34-43, 45, 47), proteins (30, 33), and other developed using copper (52), divalent metal ions (48), metal nanoparticles (41), and multiple gold nanoparticle tags (35). Among the electrochemical sensing techniques, stripping voltammetric (50, 51) and

potentiometric (41, 45, 47) techniques have been mainly used in most of the developed sensors for single (34), or dual (37) analytes.

1.2.3. The Importance of Nucleic Acid Constituents:

Nucleic acid constituents, especially adenine, guanine, adenosine, and guanosine are well-known for their metabolic and biological effects in human system. Their detection and determination has become increasingly important in the field of biomedical research owing to their importance in normal cellular functions (12). The importance of such determination stems from the fact that changes in the concentration of these compounds in body fluids have been used to indicate the presence of various diseases (53, 54). For example, adenine and guanine are involved in cellular energy homeostasis and signaling mediated by enzymatic oxidation reactions (55). In addition adenine is important in the process of genetic information transfer and it is involved in the transfer of energy, charge, and biological important compounds (56). Adenosine has potent biological activities including extension of the blood vessels, increment of the blood flow of the arteries (57), anti-arrhythmia, improvement of the oxygen supply of cardiac muscle (58); also some evidences indicate that it has some important functions in the immune system (59). Guanosine plays a protective role during brain ischemia and mediates the process of RNA splicing (12). It also can be used as the natural antioxidants and radio protectors to lethal doses of radiation and the treatment of diseases like epilepsy (60). Moreover, the levels of adenosine and guanosine in urine have been shown to be related to carcinoma and as the marker in liver diseases (61, 62).

Therefore, determination of these compounds is important for physiological and pharmacological studies, and the analysis of them has a great significance to biosciences and clinical diagnosis, so it is vital to establish simple, fast and sensitive methods for nucleic acid constituents detection.

1.3. Carbon Composite Electrode Surfaces:

Carbonaceous materials have many desirable properties that have attracted their use in electrochemistry especially for electrodes and other cell component for batteries, and these properties are good electrical conductivity, acceptable corrosion resistance, availability in high purity, high thermal conductivity, dimensional and mechanical stability, light in weight and ease of handling, availability in a variety of physical structures, ease of fabrication into composite structures, low cost.

Many forms of carbon are suitable for electroanalysis such as glassy carbon and graphite carbon, etc.

Literature survey reveals that there are only few reports using carbon electrodes for analytical determination of purine bases and their nucleosides.

1.3.1. Glassy Carbon Electrode:

Glassy carbon, also called vitreous carbon, can be fabricated as different shapes, sizes and sections, is a non-graphitizing carbon which combines glassy and ceramic properties with those of graphite. The most important properties are high temperature resistance, hardness, low density, low electrical resistance, low friction, low thermal resistance, extreme resistance to chemical attack and impermeability to gases and liquids

(63). Glassy carbon is widely used as an electrode material in electrochemistry, as well as for high temperature crucible and as a component of some prosthetic devices.

The voltammetric behavior of a glassy carbon electrode is also known to change depending upon the treatment received by the electrode surface prior to use. In general, most glassy carbon electrodes polished by using sub-micron alumina powder.

Yao et al. have reported the simultaneous determination of purine bases and their nucleosides at glassy carbon electrode (GCE) (64).

1.3.2. Graphite Pencil Electrode:

Renewable graphite pencil writing devices have been available for many years. A few years ago, the pencil lead has been used in electroanalytical chemistry as a material for electrodes. For example, it has been used in anodic stripping voltammetric measurements of trace metals (65) and for adsorptive stripping potentiometry of nucleic acids (43), also as a renewable biosensor for label-free electrochemical detection of DNA hybridization (47).

One of the advantages of graphite pencil electrode (GPE) is that it can be extruded to different lengths to yield different surface areas which make this electrode very suitable for many applications. Usually a pentel pencil (Japan) is used as a holder for the pencil lead. Electrical contact with the lead is achieved by soldering a metallic wire to the metallic part that holds the lead in place inside the pencil, (Figure 1.10A).

1.3.3. Carbon Paste Electrode:

A carbon-paste electrode (CPE) is made from a mixture of conductive graphite powder and a pasting liquid, (Figure 1.10B). These electrodes are simple to make and

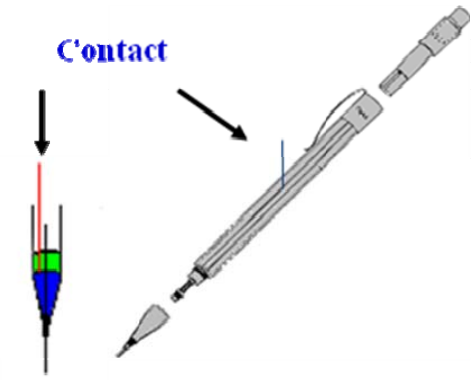
offer an easily renewable surface for electroexchange. Carbon paste electrodes belong to a special group of heterogeneous carbon electrodes. These electrodes are widly used mainly for voltammetric measurements.

Carbon pastes are easily obtainable at minimal costs and are especially suitable for preparing an electrode material modified with admixture of other compounds (66).

1.3.4. Glassy Carbon Paste Electrode:

Glassy carbon paste electrode (GCPE) is a new carbon composite electrode material based on mixing glassy carbon (GC) microparticles with mineral oil. The glassy carbon paste electrode has the electrochemical properties of glassy carbon with the advantages of composite electrodes. Glassy carbon pastes (GCPs) have many advantages such as high electrochemical reactivity, a wide accessible potential window, a low background current, inexpensive, easy to prepare, modify, and renew. Comparing with the conventional carbon pastes (CPs), the new material has a lower double-layer capacitance and a higher heterogeneous rate constant. Using scanning electron microscopy (SEM), significant differences in the structure of glassy carbon paste electrode and carbon paste electrode can be observed, Figure 1.11 shows their images (44).

(A)



(B)

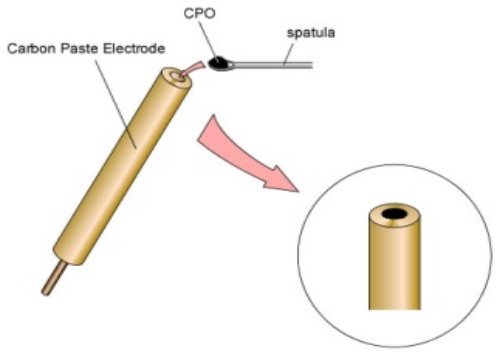


Figure 1.10. *Schematic diagram of pencil electrode (A) and carbon paste electrode (B).*

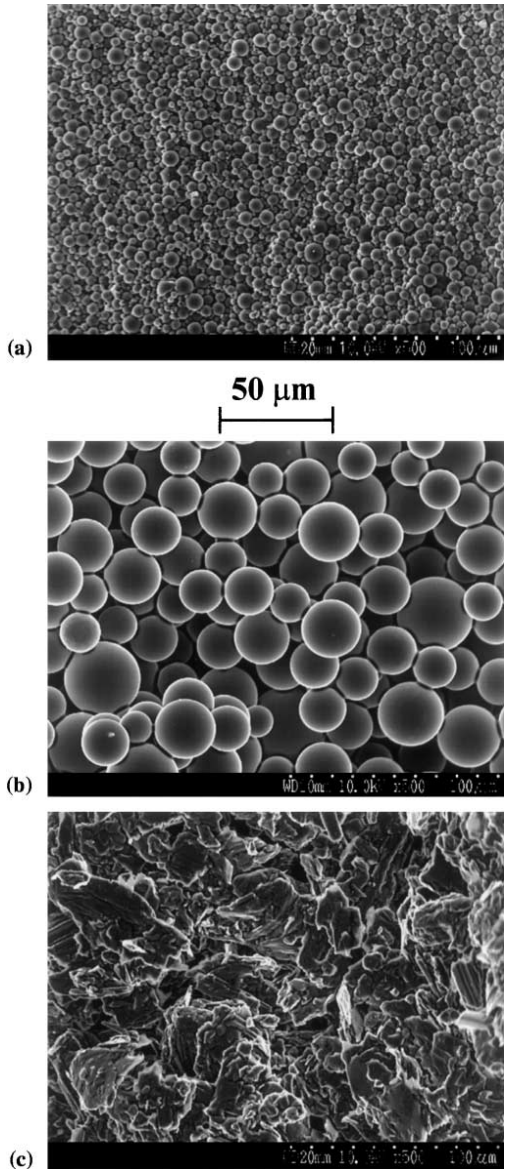


Figure 1.11. *SEM images of the GCP (a, b) and CP (c) electrodes. (a) GCP based on 0.4-12 μ m particles, (b) GCP with 20-50 μm, and (c) CP (44).*

1.4. Objectives

The overall objectives of this research are to investigate electrochemically and determine analytically few nucleic acid constituents moieties at different carbon composite electrode surfaces, where the specific objectives are as follows:

- Develop different electrochemical transducers for nucleic acid constituents analysis, particularly for adenine, adenosine, guanine and guanosine.
- Optimize the developed transducers for each one.
- Determine these compounds in pairs and simultaneously using the developed transducers.
- Investigate nucleic acid constituents electrochemically.
- Determine them in pure and biological samples (e.g. human urine).
- Compare the developed electroanalytical methods with another analytical technique (e.g. HPLC).

CHAPTER 2

2. Electrochemical Investigation and Analytical Determination of Adenine and Adenosine

2.1. Introduction

Nucleic acid constituents produce oxidation signals at mercury electrode due to the formation of sparingly soluble compounds (67, 68). Also by using different carbon electrodes, nucleic acid constituents produce anodic signals (69, 70). These signals are related to the oxidation reactions take place at the surface of the electrodes.

The objective of this chapter is to introduce different carbon electrode materials for adenine and adenosine determination by investigation, systematically, the parameters influencing their anodic response.

2.2. Electrochemical Investigation and Analytical Determination of Adenine

2.2.1.Using Glassy Carbon Paste Electrode Surfaces

2.2.1.1. Apparatus

Voltammetry measurements were performed with an electrochemical work station (CHI660C, CH Instruments Inc, Austin, TX, USA). The Ag/AgCl reference electrode (in 3M KCl, CHI111, CH Instruments Inc), and platinum wire counter electrode (CHI115, CH Instruments Inc) were inserted into the 1.0 ml glass cell through holes in its Teflon cover.

2.2.1.2. Electrode Preperation

The glassy carbon paste electrode (GCPE) was prepared by hand-mixing 70 mg of 20-50 μm glassy carbon powder with 30 mg of mineral oil. The portion of the resulting paste was then packed firmly into the electrode cavity (1.0 mm diameter and 2.0 mm depth) of the PTFE sleeve. Electrical contact was established via a copper wire. The paste surface was smoothed with a weighing paper, and then rinsed carefully with deionized water prior to each measurement.

2.2.1.3. Reagents

Adenine, sodium acetate and phosphate buffer solutions with different pHs, that were served as the supporting electrolyte, were obtained from Sigma (St. Louis, Mo, USA). Glassy carbon spherical powders (20 – 50 μm) were obtained from Alfa Aesar (Ward Hill, MA, USA). The mineral oil was obtained from Aldrich. All stock solutions were prepared using deionized water. Copper atomic absorption standard solutions (containing 1000 μg/ml Cu(II), with 0.5 mol/l HNO_3), cadmium atomic absorption standard solutions (containing 1000 μg/ml Cd(II), with 0.5 mol/l HNO_3), lead atomic absorption standard solutions (containing 1000 μg/ml Pb(II), with 0.5 mol/l HNO_3) were purchased from Aldrich. Mannitol and EDTA was obtained from BDH Analar (Poole, England), lactose was purchased from B.B.L (Baltimore, Maryland, USA), sodium dodecyl benzene sulfonate was purchased from Science lab.com (Houston, Texas, USA), while ascorbic acid was obtained from Fluka AG (Buchs, Switzerland).

2.2.1.4. Procedure

Cyclic voltammetric (CV) measurements were performed by treating the surface of the electrode at +1.7 V for 60s followed by 120s accumulation at +0.2 V in a stirred solution of 0.2 M phosphate buffer (pH7.0) (or as mentioned otherwise) containing a specific concentration of adenine. The stirring was then stopped for 5s; before scanning the potential at 100 mV/s (or as mentioned otherwise) for one or more complete cycle within the potential range -0.2 to +1.6 V (vs. Ag/AgCl. Sat. KCl) reference electrode.

Using square wave voltammetry (SWV), measurements were performed by treating the surface of the electrode at a potential of +1.7 V for 60s followed by 120s accumulation at +0.2 V in a stirred solution of 0.2 M phosphate buffer (pH7.0) containing a specific concentration of adenine. The stirring was then stopped for 5s quiet time; followed by a subsequent stripping using a square wave voltammetric waveform, with a 4 mV potential step, 100 Hz frequency and amplitude of 50 mV (or as mentioned otherwise). A new glassy carbon paste surface was used in every measurement. The electrode surface was smoothed and rinsed carefully with deionized water prior to every measurement.

2.2.1.5. Results and Discussion

The present study evaluates the use of glassy carbon paste composite material as a new material/transducer for adenine analytical determination.

2.2.1.5.1. Electrochemical Investigation

Cyclic voltammetry is considered as one of the best techniques available used to investigate electroactive analytes electrochemicaly. In this part the electrochemical behaviour of adenine using cyclic voltammetric technique, was investigated.

Figure 2.1 displays cyclic voltammogram of 1.0 ppm adenine obtained at 100 mV/s using GCPE. By scanning the potential from -0.2 to +1.6 V, one oxidation peak appears at +0.99V for adenine with an irreversible oxidation process.

Effect of scan rate (U) has been studied on the response of adenine as showen in Figure 2.2, the electrochemical response of 1.0 ppm adenine at scan rate 25, 50, 75, 100, 150, and 200 mV/s were obtained. Peak current versus (U) were plotted in Figure 2.2B. It has been noticed that the signal increases linearly up to 150 mV/s and then levels off.

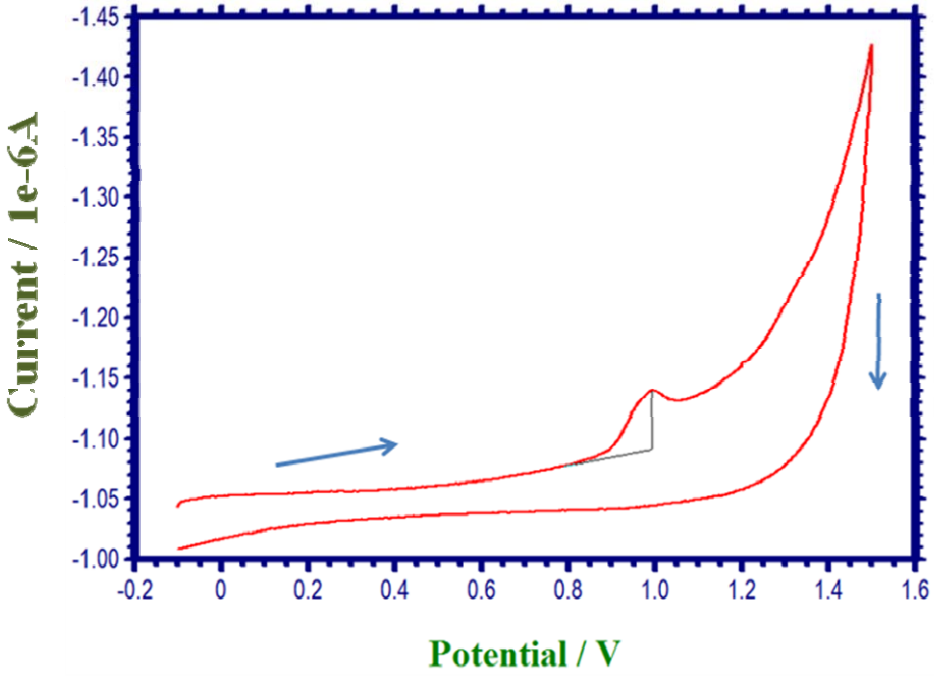


Figure 2.1. *Cyclic voltammogram of 1.0 ppm adenine using GCPE at scan rate 100 mV/s. Measurements were performed in phosphate buffer solution (0.2 M, pH7.0). Electrode pretreatment 1.0 min at +1.7 V. Accumulation time 2.0 min at +0.2V.*

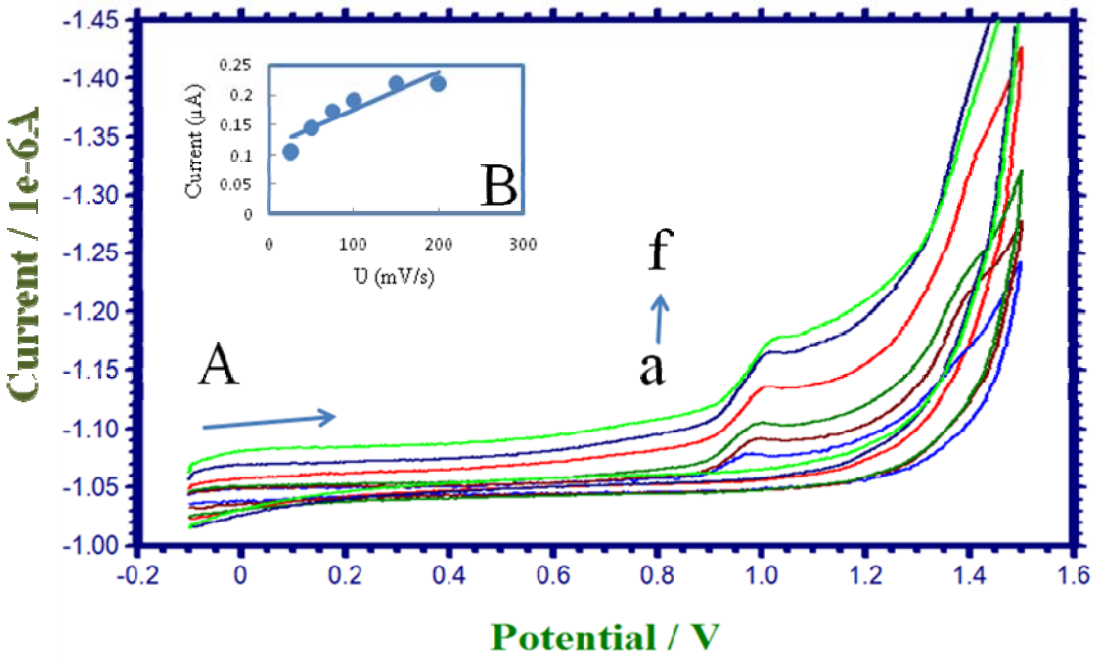


Figure 2.2. *(A) Cyclic voltammograms of 1.0 ppm adenine using GCPE at different scan rates; 25(a), 50(b), 75(c), 100(d), 150(e), and 200(f) mV/s. (B) The corresponding plot. Other condition, as in Figure 2.1.*

2.2.1.5.2. Optimization

To find out the best detection medium from buffer composition and pH, both acetate and phosphate buffers with different pHs were used as shown in Figure 2.3.

Figure 2.3 shows the square-wave stripping voltammetric responses of 1.0 ppm adenine at the fabricated glassy carbon paste electrode (GCPE). The adenine electrochemical response in phosphate medium was more predominate from the response in acetate as shown in Figure 2.3 (a, b vs. e, c and d). There is a cathodic potential shift for the adenine oxidation peak position with the increase of the pH. Within the same phosphate medium, this indicated that protons were involved in the electrode reaction. A good linear relationship was established between the oxidation peak potential and the solution pH with the linear regression equation as:

$$E_{pa}\,(V) = -0.057pH + 1.297 \qquad (R^2 = 0.974)\ldots\ldots\ldots\ldots\ldots\ldots \text{(Eq. 2.1)}$$

pH 7.0 was the optimum pH among all examined pHs with respect to the height and shape of the obtained adenine oxidation peak.

The dependence of the square wave voltammetry oxidation peaks of adenine was studied at different pulse amplitude as shown in Figure 2.4. The Figure 2.4 shows typical square-wave stripping voltammograms of 1.0 ppm adenine where measurements were performed in phosphate buffer solution (0.2 M, pH7.0). From the obtained voltammograms, and corresponding plot, Figure 2.4B, the height of the peak increases

with increasing the amplitude up to 50 mV then decreases thereafter. For that 50 mV pulse amplitude was chosen as an optimum.

Adenine peak at different frequencies was carried out as shown in Figure 2.5. The electrochemical responses at different frequencies were obtained and peak currents were plotted in the Figure 2.5B. As can be seen, the larger the frequency, the higher the oxidation peaks. Taking in consideration the noise generated at frequencies higher than 100 Hz, a frequency of 100 Hz was used on the subsequent experiments.

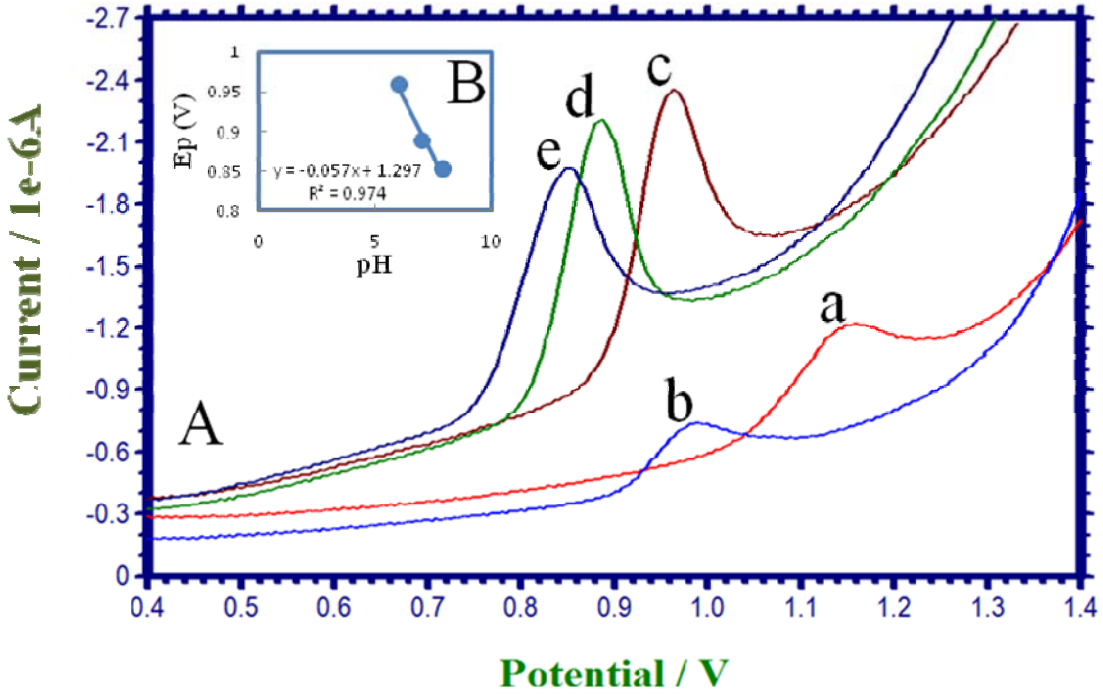


Figure 2.3. *(A) Square-wave stripping voltammograms of 1.0 ppm adenine using GCPE in different buffer media with different pHs: 0.2M acetate buffer; pH 4.0 (a), and pH 6.0 (b); 0.2 M phosphate buffer; pH 6.0 (c), pH 7.0 (d), and pH 7.9 (e). Electrode pretreatment, 1.0 min at +1.7 V; Accumulation time, 1.0 min at +0.5 V. Potential step, 4 mV; Frequency, 25 Hz; Pulse amplitude, 50 mV. (B) The corresponding plot of the adenine oxidation potential and the phosphate solution pH.*

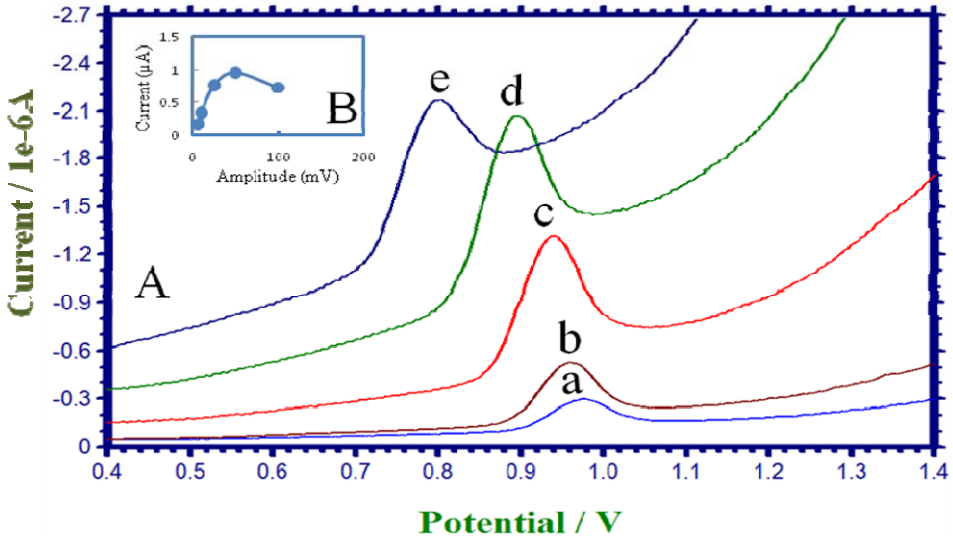


Figure 2.4. *(A) Square-wave stripping voltammograms for 1.0 ppm adenine using GCPE at different pulse amplitude: 5.0 (a), 15 (b), 25 (c), 50 (d) and 100 (e) mV. Measurements were performed in phosphate buffer solution (0.2 M, pH7.0). (B) The corresponding plot. Other conditions, as in Figure 2.3.*

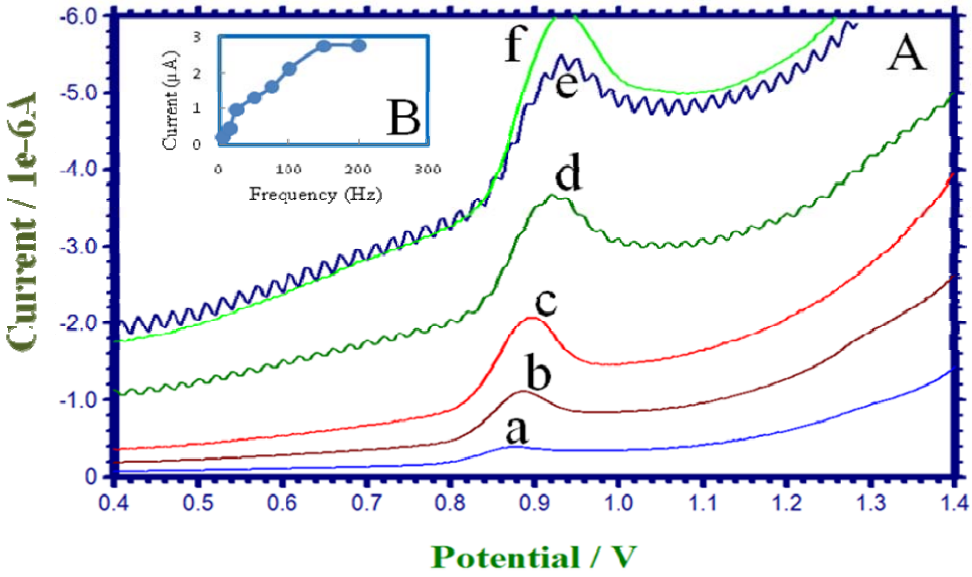


Figure 2.5. *(A) Square-wave stripping voltammograms of 1.0 ppm adenine using GCPE at different frequency: 5.0 (a), 15 (b), 25 (c), 50 (d), 75 (e) and 100 (f) Hz. (B) The corresponding plot. Other conditions, as in Figure 2.3.*

Both accumulation time and accumulation potential were studied as well. Figure 2.6 shows the optimization of the accumulation potential of 1.0 ppm adenine, where the potential was varied from 0.0V to +1.0 V (vs. Ag/AgCl), Figure 2.6 reflects the influence of the accumulation potential on the response of 1.0 ppm adenine, the signal increase slowly upon changing the potential between 0.0 and +0.2, start decreasing after that. Accumulation potential of +0.2 V yielded the best tradeoff between the sensitivity, background contribution and corresponding noise, and was thus used for all subsequent work.

After different accumulation times the responses of 1.0 ppm adenine were recorded. Figure 2.7 shows the square-wave stripping voltammograms of 1.0 ppm adenine after accumulation times; 30, 60, 120 and 300 sec, where the signal increases up to 120s then starts decreasing. The corresponding plot, Figure 2.7 B, supports that the 120 sec is the optimum accumulation time for the adenine detection under the conditions. Such profile could be due to the possible surface saturation of glassy carbon paste with the adenine molecules.

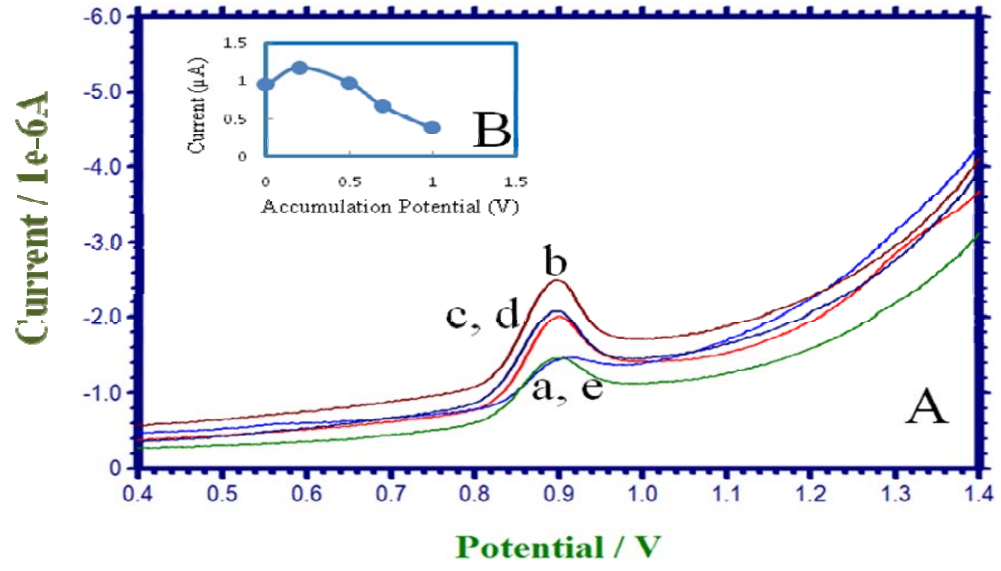


Figure 2.6. *(A) Square-wave stripping voltammograms for 1.0 ppm adenine using GCPE at different accumulation potential: 0.0(a), 0.2(b), 0.5(c), 0.7(d) and 1.0(e) V. Measurements were performed in phosphate buffer solution (0.2 M, pH7.0). (B) The corresponding plot. Other conditions, as in Figure 2.3.*

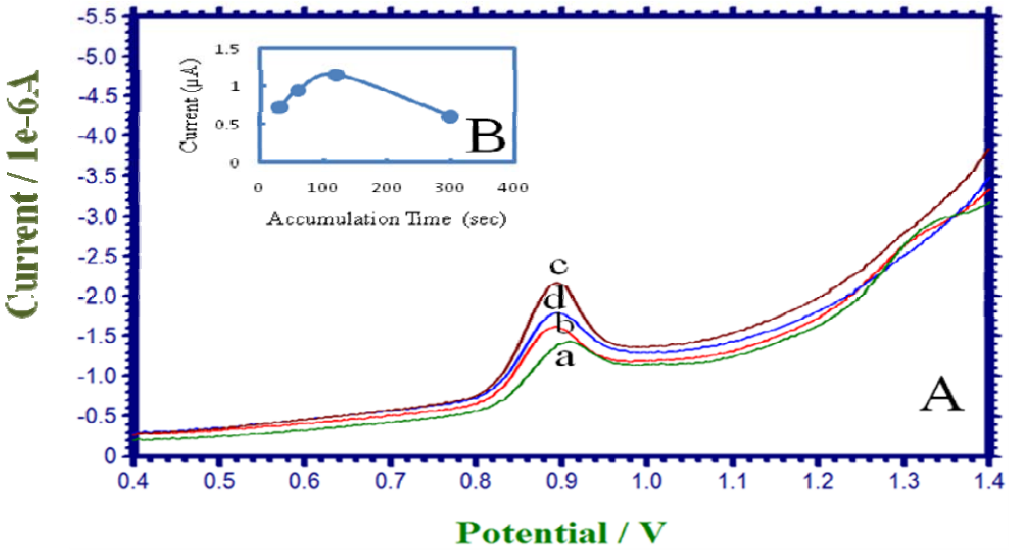


Figure 2.7. *(A) Square-wave stripping voltammograms of 1.0 ppm adenine using GCPE at different accumulation times: 30(a), 60(b), 120(c) and 300(d) sec. Measurements were performed in phosphate buffer solution (0.2 M, pH7.0). (B) The corresponding plot. Other conditions, as in Figure 2.3.*

2.2.1.5.3. Analytical Determination

Figure 2.8 shows the calibration study under these optimum conditions. With the increase of the adenine concentration, the peak current increases linearly within the concentration range 0.1 to 4.0 ppm. Further increase in the adenine concentration deviates from linearity as shown in the Figure 2.8B. This is due to a possible complete coverage of the electrode surface as the concentration increases.

Measurements of a 50 ppb adenine solution were used to estimate the detection limit. A value of around 17.0 ppb can thus be estimated based on the signal-to-noise characteristics of these data (S/N = 3). Such detection limit corresponds to 16.6 ppb (0.125 μmol/l). A series of seven measurements of 1.0 ppm adenine yielded a highly reproducible response with a relative standard deviation of 0.1304.

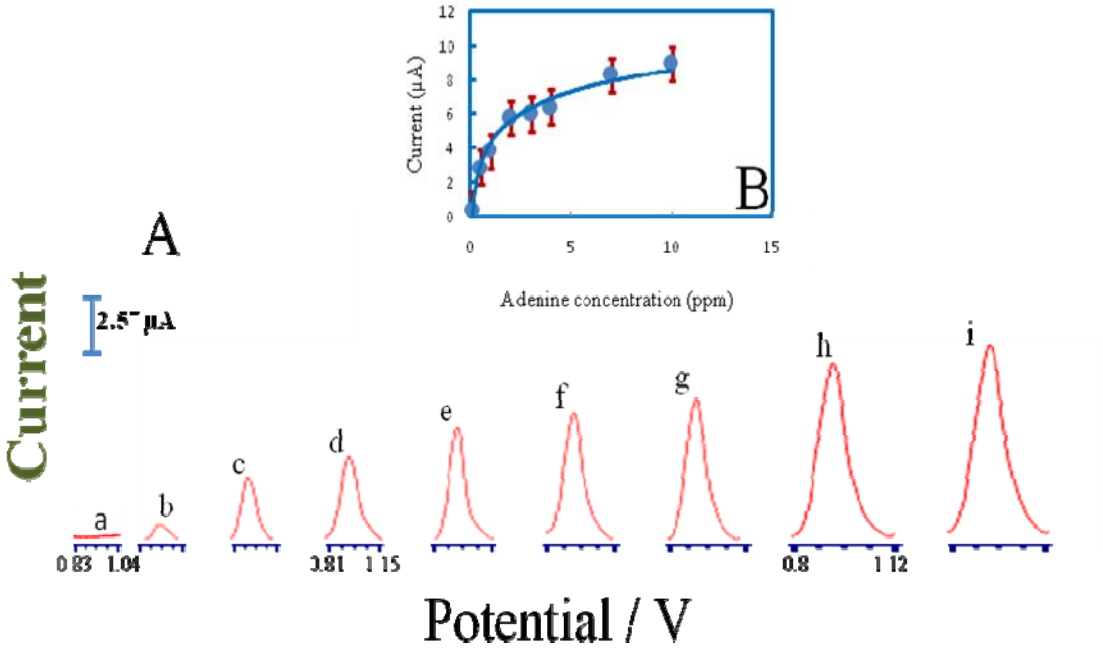


Figure 2.8. *(A) Square-wave stripping signals of adenine at GCPE: 0.0 (a), 0.1 (b), 0.5 (c), 1.0 (d), 2.0 (e), 3.0 (f), 4.0 (g), 7.0 (h), and 10 (i) ppm. Measurements were performed in phosphate buffer solution (0.2 M, pH7.0). Electrode pretreatment, 1 min at +1.7 V; Accumulation time, 2.0 min at +0.2V. Potential step, 4 mV; Frequency, 100 Hz; Pulse amplitude, 50 mV. (B) The corresponding calibration plot.*

2.2.1.5.4. Interferences

As we have mentioned above, nucleic acid constituents interact with metal ions and stabilize or destabilize the double helix (23). The organic compounds can affect the oxidation signal of nucleic acid constituents as well.

Shiraishi and Takahashi reported on the enhanced voltammetric response of free (monomeric) guanine and adenine nucleobases in the presence of copper, and attributed their observation to accumulation of the copper(I)–purine complex (70).

We have studied in this section the interference effect of heavy metals (Cu, Cd, Pb) and some organic compounds such as ascorbic acid, lactose, mannitol, EDTA and sodium dodecylbenzene sulfonate (SDBS) on the adenine response.

The effect of copper ions, Cu(II), as a heavy metal, is shown in Figure 2.9. This Figure compares square wave voltammograms at glassy carbon paste electrode for 1.0 ppm adenine in absence (a) and in presence (b) of 2.0 ppm copper ions, Cu(II). A substantial enhancement of the adenine peak with a shift to a higher potential is observed in the presence of copper ions.

The influences of adding different concentratoins of different organic compounds on the determination of 2.0 ppm adenine were studied and the results are shown in Table 2.1. Most of the interferences including ascorbic acid, lactose and mannitol at the concentration of 1.0 ppm caused less than 10% current signal changes, indicating that the proposed method has a good selectivity for adenine detection.

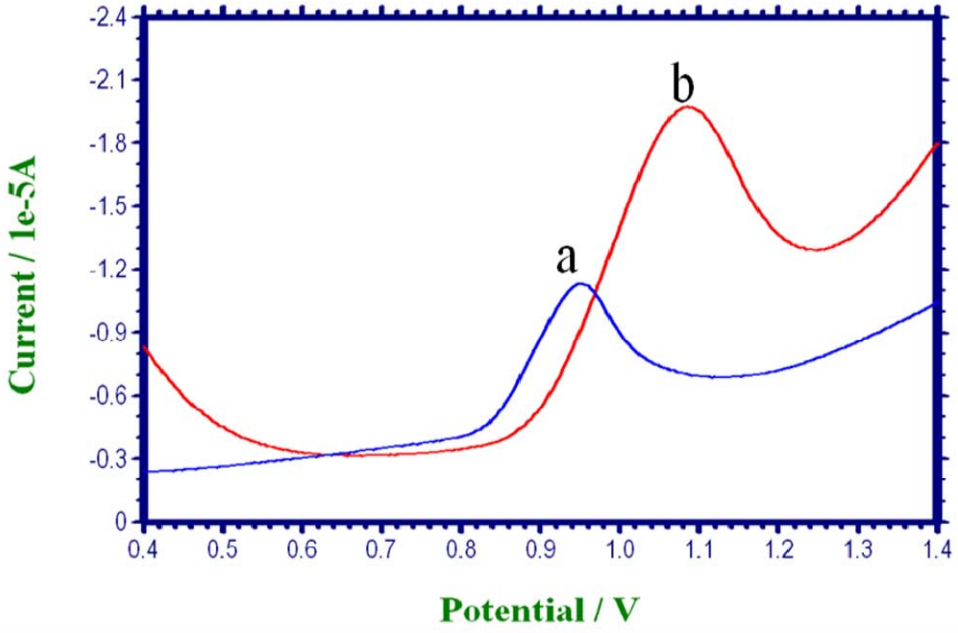


Figure 2.9. *Square-wave stripping voltammograms of 1.0 ppm adenine in absence (a) and in presence (b) of 2.0 ppm Cu(II) using GCPE. Measurements were performed in phosphate buffer solution (0.2 M, pH7.0). Electrode pretreatment, 1.0 min at +1.7 V; Accumulation time, 2.0 min at -0.05V. Potential step, 4 mV; Frequency, 100 Hz; Pulse amplitude, 50 mV.*

Table 2.1. Influence of interferences on the determination of 2.0 ppm adenine.

Interference	Concentration/ppm	Change of Signal (%)
Ascorbic Acid	1	10.0
	3	20.1
	5	28.3
Lactose	1	0.1
	3	13.6
	5	35.3
sodium dodecylbenzene sulfonate	1	16.4
	3	31.09
	5	57.08
Mannitol	1	7.2
	3	13.8
	5	30.9
EDTA	1	13.74
	3	30.25
	5	53.02

2.2.1.5.5. Application

The developed detection method using the glassy carbon paste composite material was tested on the detection of adenine in human urine medium. The urine sample was diluted 1:10 (by volume) with deionized water. In a micro centrifuge tube, 10 µl of 100 ppm of adenine solution was added to 1.0 ml of the diluted urine. The final adenine concentration added to the sample was 1.0 ppm.

Representative square-wave stripping voltammograms for trace determination of adenine (1.0 ppm) spiked in human urine were illustrated in Figure 2.10, where a defined adenine signal was obtained as shown in Figure 2.10b, corresponding to the 1.0 ppm adenine concentration.

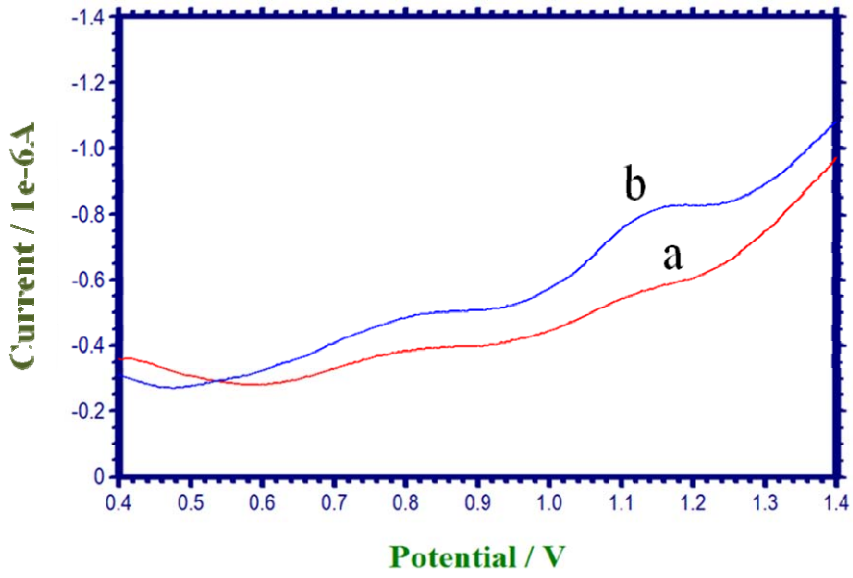


Figure 2.10. *Square-wave stripping voltammograms of 1.0 ppm adenine using GCPE in Urine medium. Other conditions, as in Figure 2.9.*

2.2.2. Using Glassy Carbon Electrode Surfaces

Many electrodes can be used for the analysis of nucleic acid constituents. Some work has been reported with gold (71), platinum, copper, and silver (72) solid electrodes. In this study we focus on the carbon electrodes. Different carbon electrodes were used as transducers. Glassy carbon electrode (GCE) and graphite pencil electrode (GPE) are among these electrodes.

In this section the electrochemical investigation and analytical determination of adenine at glassy carbon electrode will be discussed.

2.2.2.1. Electrode Preparation

Glassy carbon electrode (GCE) 3.0 mm diameter, was polished with 0.3 μm alpha alumina powder and rinsed with deionized water before each measurement.

2.2.2.2. Procedure

For glassy carbon electrode, polishing with alumina powder and rinsing with deionized water should be done before each measurement. After accumulation at +0.2 V in a stirred solution of phosphate buffer (0.2 M, pH7.0) containing a specific concentration of adenine, the stirring was stopped for 5s. This was followed by a subsequent stripping using a square wave voltammetric waveform, with a 4 mV potential step, 25 Hz frequency and amplitude of 50 mV.

2.2.2.3. Results and Discussion

2.2.2.3.1. Electrochemical Investigation

As expected one oxidation peak due to an irreversible process appeared when cyclic voltammetry was used to investigate the adenine at glassy carbon electrode electrochemically. Figure 2.11 shows the obtained cyclic voltammogram.

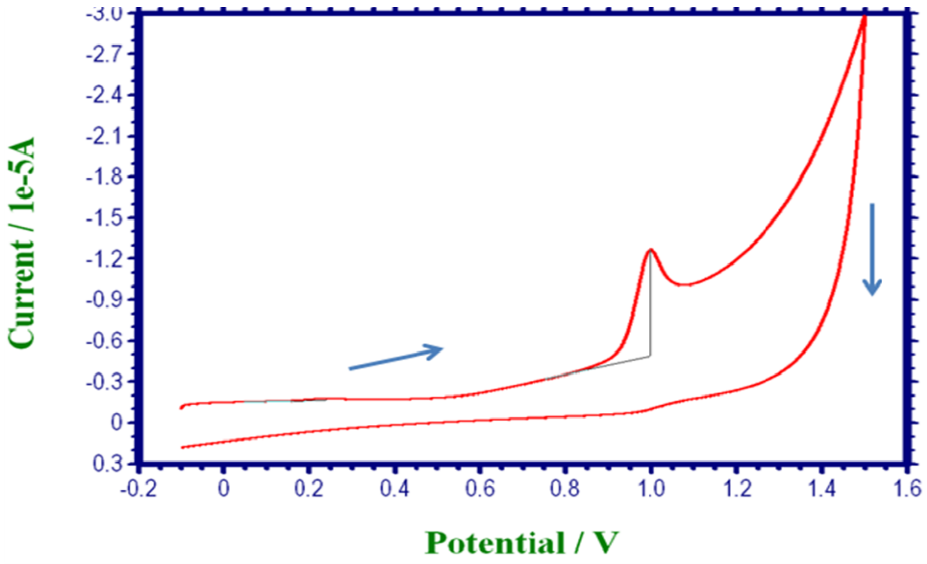


Figure 2.11. *Cyclic voltammogram of 5.0 ppm adenine using GCE at scan rate 100 mV/s. Measurements were performed in phosphate buffer solution (0.2 M, pH7.0). Accumulation time, 30 sec at +0.2V.*

2.2.2.3.2 Optimization

Parameters related to the square wave voltammetric technique were optimized at glassy carbon electrode surfaces.

Figure 2.12A, shows the influence of applying different pulse amplitudes on the SWV adenine response. As can be seen in corresponding plots Figure 2.12B, the adenine response increases rapidly up to 25 mV then slowly till 50 mV after that starts decreasing. So 50 mV was chosen as an optimum parameter.

Affect of frequency and accumulation time were examined as well. Figure 2.13A, displays the effect of frequency on the height of adenine peak, as shown, the larger the frequency, the higher the peak. But with consideration for the shape of the obtained adenine peak, we have noticed that at frequencies higher than 25 Hz, the obtained signal becomes noisy. For that a frequency of 25 Hz was chosen as the optimum one.

According to the obtained accumulation time results, where 30 sec gave the highest response, the response starts decreasing gradually after that, as shown in Figure 2.13B. By comparing with similar ones at glassy carbon paste electrode, we realize that the accumulation time effect using GCE is much less than in GCPE and this could be due to the different morphology of both electrode surfaces.

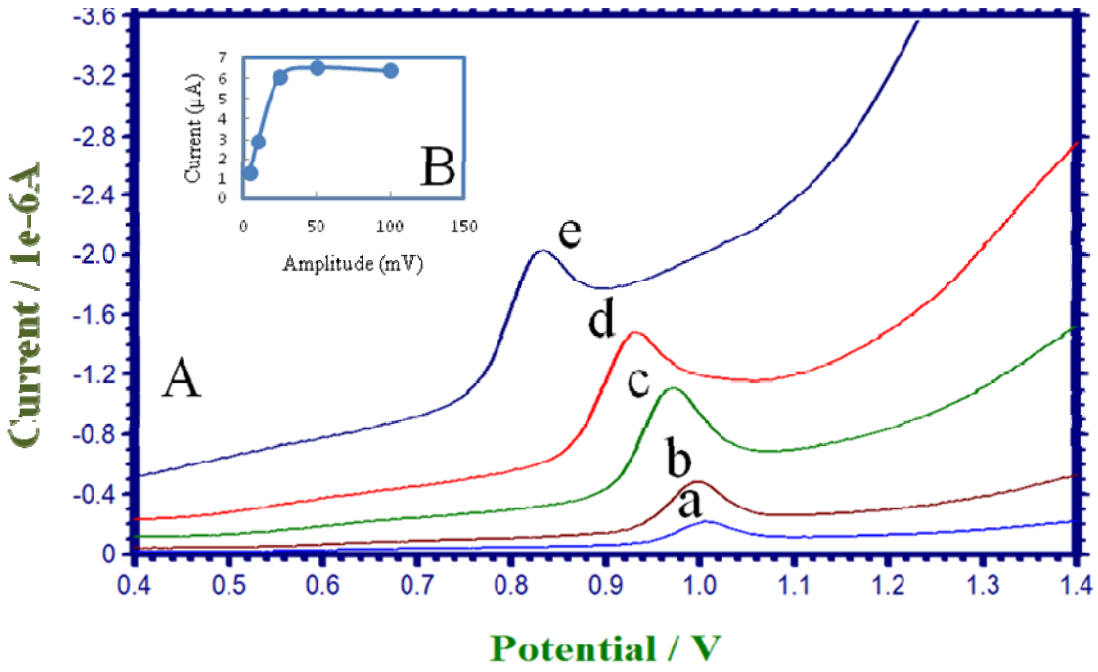


Figure 2.12. *(A) Square-wave stripping voltammograms 0f 5.0 ppm adenine using GCE at different pulse amplitude: 5.0 (a), 10 (b), 25 (c), 50 (d) and 100 (e) mV. Measurements were performed in phosphate buffer solution (0.2 M, pH7.0). Accumulation time, 30 sec at +0.5 V. Potential step, 4 mV; Frequency, 25 Hz; (B) The corresponding plot.*

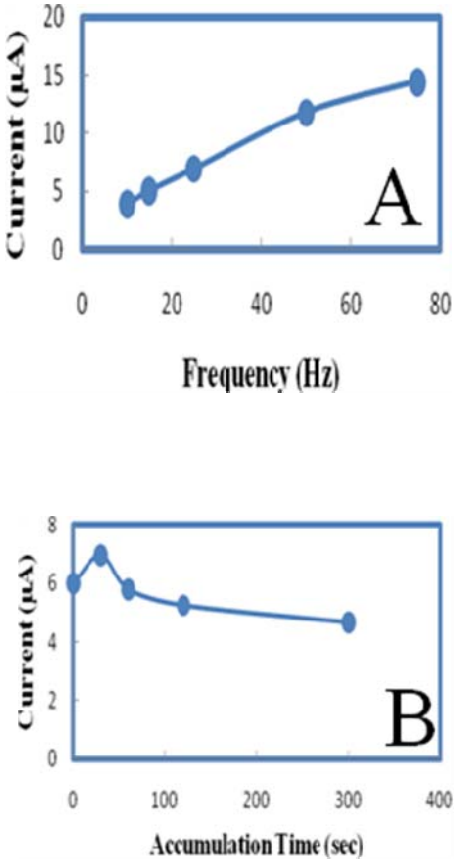


Figure 2.13. *Effect of frequency (A) and accumulation time (B) upon the square-wave stripping voltammetric response of 5.0 ppm adenine using GCE. Pulse amplitude, 50 mV. Other conditions, as in Figure 2.12.*

2.2.2.3.3 Analytical Determination

Under the optimum conditions, the square wave stripping voltammetric responses were obtained at different adenine concentrations using glassy carbon electrode as shown in Figure 2.14.With increasing the adenine concentration from 0.5 – 12 ppm, the peak current increases linearly as shown in Figure 2.14B. Further increase in the concentration causes a marked non-linear influence on the peak current. This is could be due to a nearly total coverage and saturation of the glassy carbon electrode surface.

To calculate the relative standard deviation (RSD) seven measurements were recorded for different 5.0 ppm adenine samples as shown in Figure 2.15. The calculated RSD is 0.0532, which reflects the good reproducibility of the developed analytical method.

Using GCE the detection limit was 100 ppb (0.74 μmol/l) which is much less than using GCPE.

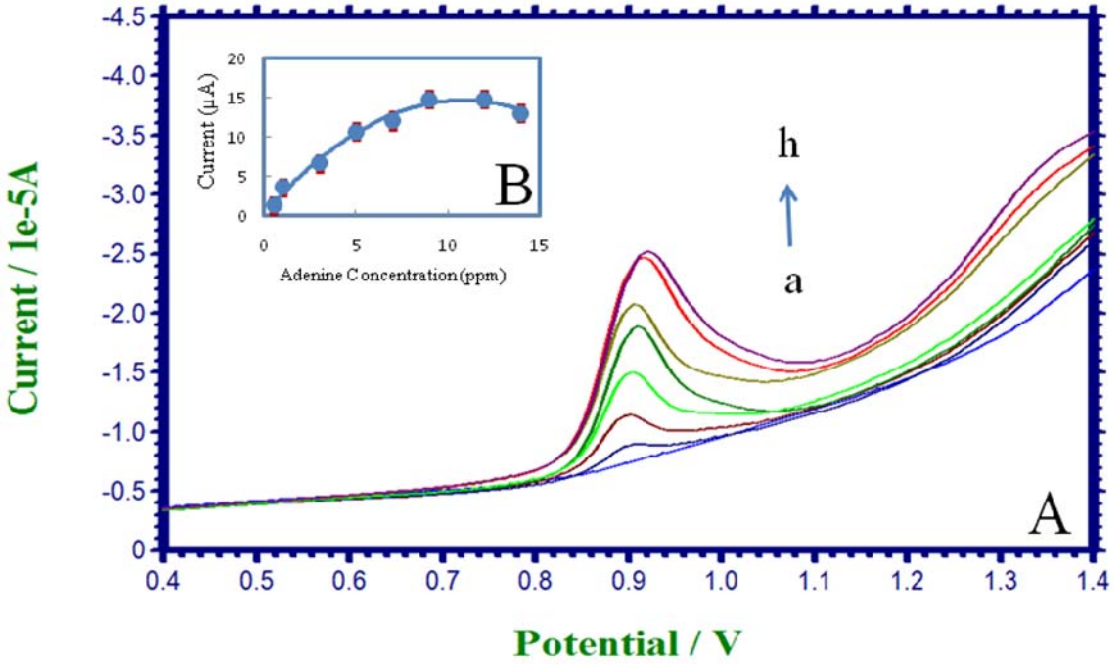


Figure 2.14. *(A) Square-wave stripping voltammograms of adenine concentrations using GCE : 0.0 (a), 0.5 (b), 1.0 (c), 3.0 (d), 5.0 (e), 7.0 (f), 9.0 (g) and 12 (h) ppm. Measurements were performed in phosphate buffer solution (0.2 M, pH7.0). Accumulation time, 30 sec at +0.2V. Potential step, 4 mV; Frequency, 25 Hz; Pulse amplitude, 50 mV. (B) The corresponding calibration plot.*

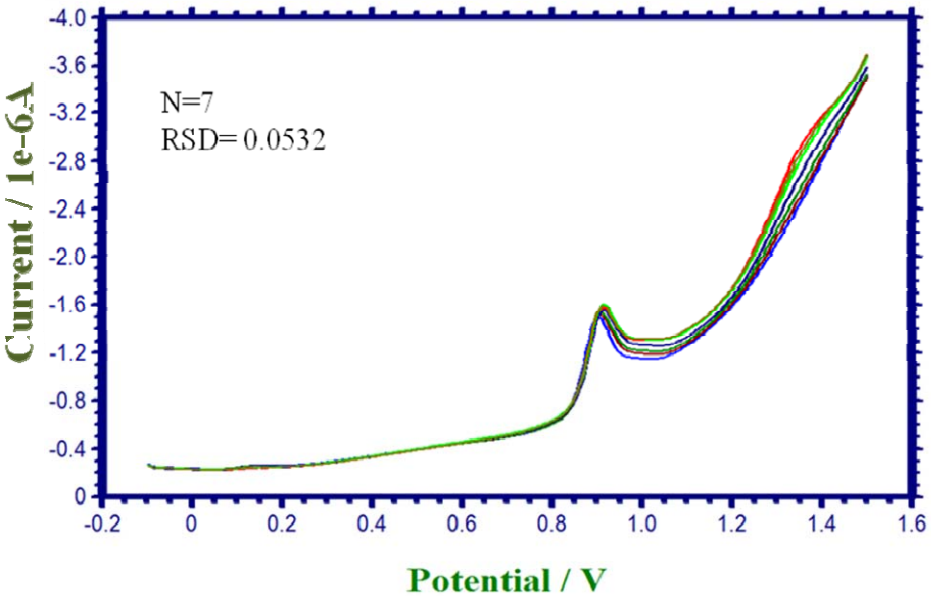


Figure 2.15. *Square-wave stripping voltammograms of 5.0 ppm adenine using GCE. A series of measurements of seven different adenine samples and a new surface every time. Measurements were performed in phosphate buffer solution (0.2 M, pH7.0). Other conditions, as in Figure 2.14.*

2.2.3. Using Graphite Pencil Electrode Surfaces

2.2.3.1. Electrode Preparation

A pencil Model P205 (Pentel, Japan) was used as a holder for the pencil lead. Electrical contact with the lead was achieved by soldering a metallic wire to the metallic part that holds the lead in place inside the pencil. The pencil was fixed vertically with 11 mm of the pencil extrude outside and 6.0 mm of the lead that was immersed in the solution. Such length corresponds to an active electrode area of about 9.82 mm^2.

2.2.3.2. Procedure

The pencil was fixed vertically with 11 mm of the pencil extrude outside and 6.0 mm of the lead that was immersed in the solution. A short ,30 s, electrochemical pre-treatment at +1.4 V (using the blank phosphate buffer solution; 0.2 M, pH 7.0) preceded the measuring step 60s accumulation at +0.2 V in a stirred solution of phosphate buffer (0.2 M, pH7.0) containing a specific concentration of adenine. The stirring was then stopped for 5s; this was followed by a subsequent stripping using a square wave voltammetric waveform, with a 4 mV potential step, 25 Hz frequency and amplitude of 50 mV.

2.2.3.3. Results and Discussion

2.2.3.3.1. Analytical Determination

Figure 2.16 shows the calibration plots for adenine at graphite pencil electrode. The response increase nearly linearly up to 25 ppm.

Same study was done to know the detection limit and relative standard deviation for adenine at graphite pencil electrode, and the results are the following:

Detection limit is 35 ppb (0.24 µmol/l) and the relative standard deviation is 0.6798 for seven measurements of different 5.0 ppm adenine sample solutions.

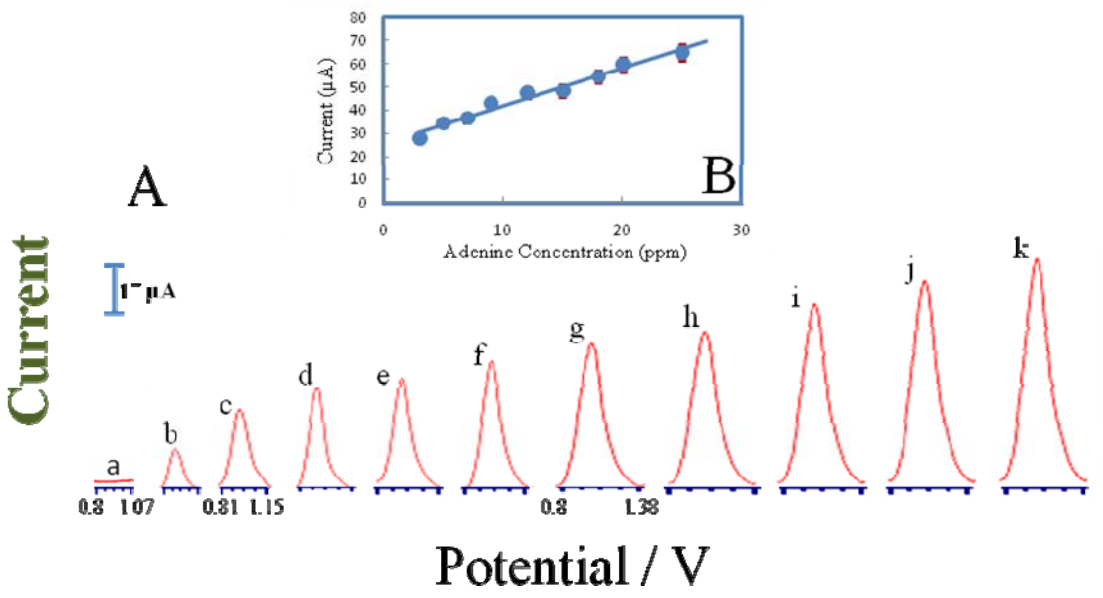


Figure 2.16. *(A) Square-wave stripping signals of adenine at GPE: 0.0 (a), 1.0 (b), 3.0 (c), 5.0 (d), 7.0 (e), 9.0 (f), 12 (g), 15 (h), 18 (i), 20 (j) and 25 (k) ppm. Measurements were performed in phosphate buffer solution (0.2 M, pH7.0). Electrode pretreatment,30 sec at +1.4 V; Accumulation time, 1.0 min at +0.2V. Potential step, 4 mV; Frequency, 25 Hz; Pulse amplitude, 50 mV. (B) The corresponding calibration plot.*

Table 2.2 summarizes the results related to detection limits for adenine at the different carbon electrodes (GCPE, GCE, and GPE) as well as the corresponding relative standard deviations which calculated after a series of seven measurements of adenine sample at each electrode.

As we can notice from the Table 2.2, glassy carbon paste electrode was the best with respect to the detection limit that is 0.125 μmol/l, while glassy carbon electrode yielded a highly reproducible response with the best relative standard deviation of 0.0532.

Table 2.2. The detection limits and relative standard deviations for adenine at glassy carbon paste (GCP), glassy carbon (GC), and graphite pencil (GP) electrodes.

Electrode	**Detection Limit (µmol/l)**	**Relative Standard Deviation**
GCPE	0.125	0.1304
GCE	0.74	0.0532
GPE	0.24	0.6798

2.3. Electrochemical Investigation and Analytical Determination of Adenosine

2.3.1.Using Glassy Carbon Paste Electrode Surfaces

2.3.1.1. Reagents

The following chemicals were obtained from Sigma, adenosine, sodium acetate and phosphate buffer solutions with different pHs, that were served as the supporting electrolyte. Glassy carbon spherical powders (20 – 50 μm) were obtained from Alfa Aesar (Ward Hill, MA, USA). The mineral oil was obtained from Aldrich. Copper atomic absorption standard solutions (containing 1000 μg/ml Cu(II), with0.5 mol/l HNO3), cadmium atomic absorption standard solutions (containing 1000 μg/ml Cd(II), with 0.5 mol/l HNO_3), lead atomic absorption standard solutions (containing 1000 μg/ml Pb(II), with 0.5 mol/l HNO_3), were purchase from Aldrich. Mannitol and EDTA was obtained from BDH Analar (Poole, England), lactose was purchased from B.B.L (Baltimore, Maryland, USA), sodium dodecyl benzene sulfonate was purchased from Science lab. Com (Houston, Texas, USA), while ascorbic acid was obtained from Fluka AG (Buchs, Switzerland). All stock solutions were prepared using deionized water.

2.3.1.2. Procedure

Cyclic voltammetry (CV) measurements were performed by treating the surface of the electrode at +1.7 V for 60s followed by 120s accumulation at +0.2 V in a stirred solution of 0.2 M phosphate buffer (pH7.0) (or as mentioned otherwise) containing a specific concentration of adenosine. The stirring was then stopped for 5s; before scanning the potential at 100 mV/s (or as mentioned otherwise) for one or more complete cycle within the potential range -0.2 - 1.6 V (vs. Ag/AgCl. Sat. KCl) reference electrode.

Using square wave voltammetry (SWV), measurements were performed by treating the surface of the electrode at +1.7 V for 60s followed by 120s accumulation at +0.2 V in a stirred solution of 0.2 M phosphate buffer (pH7.0) containing a specific concentration of adenosine. The stirring was then stopped for 5s; was followed by a subsequent stripping using a square wave voltammetric waveform, with a 4.0 mV potential step, 100 Hz frequency and amplitude of 50 mV (or as mentioned otherwise). A new glassy carbon paste surface was used in every measurement. The electrode surface was smoothed and rinsed carefully with deionized water prior to every measurement.

2.3.1.3. Results and Discussion

In the present work we examine the electrochemical investigation and the parameters affecting the analytical determination of adenosine at glassy carbon paste electrode surfaces.

2.3.1.3.1. Electrochemical Investigation

Figure 2.17 shows the cyclic voltammogram for 5.0 ppm adenosine solution, following 2.0 min accumulation at a potential of +0.2 V. As can be seen in Figure 2.17, the oxidation process happened at the electrode surface is an irreversible process with one oxidation peak at the position +1.27 V.

A substantial enhancement of the adenosine response is observed when applying different scan rates (U); Figure 2.18A, displays the effect of different scan rate on the 5.0 ppm adenosine solution, as expected the larger the scan rate, the higher the adenosine peak. That is because when the reaction is driven faster, the surface concentration of the reactant is progressively reduced. The concentration gradient is thereby increased given

by Nernst equation (Eq. 2.2) and hence its current also increases. By plotting the current vs. U, Figure 2.18 B, we notice that the response increases linearly with increasing the scan rate, which means that an adsorption phenomena takes place on the surface of the electrode.

(Total cell potential)

$$E_{\text{cell}} = E^{\ominus}_{\text{cell}} - \frac{RT}{zF} \ln Q \qquad \text{..... (Eq. 2.2)}$$

Where:

E_{cell} is the cell potential (electromotive force)

E^{o}_{cell} is the standard cell potential at the temperature of interest

R is the universal gas constant, R = 8.314 J/Kmol

T is the absolute temperature

F is the Faraday constant, the number of coulombs per mole of electrons, F = 9.648×10^4 C/mol

z is the number of moles of electrons transferred in the cell reaction or half-reaction

Q is the reaction quotient.

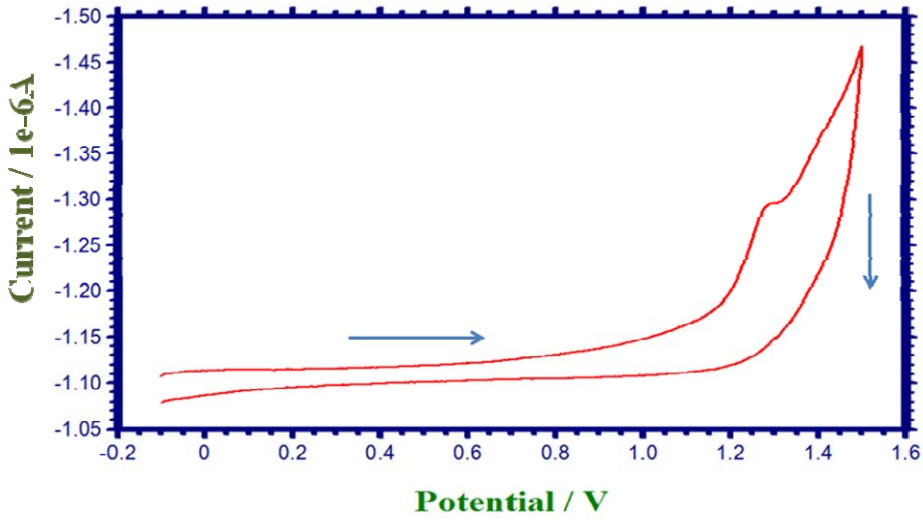


Figure 2.17. *Cyclic voltammogram of 5.0 ppm adenosine using GCPE at scan rate 100 mV/s. Measurements were performed in phosphate buffer solution (0.2 M, pH7.0). Electrode pretreatment 1.0 min at +1.7 V. Accumulation time, 2.0 min at +0.2V.*

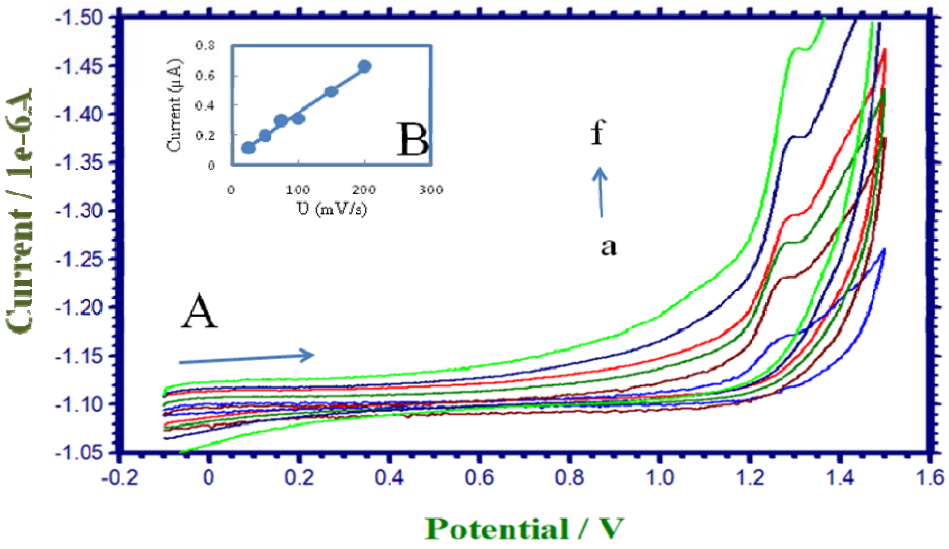


Figure 2.18. *(A) Cyclic voltammograms of 5.0 ppm adenosine using GCPE with different scan rates, 25 (a), 50 (b), 75 (c), 100 (d), 150 (e), 200 (f) mV/s. (B) The corresponding plot. Other conditions, as in Figure 2.17.*

2.3.1.3.2. Optimization

Since the electrical response of the adenosine relies on the stripping voltammetric detection, it is essential to examine and optimize relevant experimental parameters.

As shown in Figure 2.19, different buffers with different pHs were used for measuring the square-wave stripping voltammetric responses of 5.0 ppm adenosine at newly prepared glassy carbon paste electrode (GCPE) surfaces. From Figure 2.19, using phosphate buffer solution gave extremly better results (c, d, e) than that obtained using acetate buffer (a, b), and within the phosphate buffer the best adenosine response with respect to peak potential, height and shape, was obtained in pH 7.0.

The results indicated that the oxidation peak potential of adenosine shifted negatively with the increment of the solution pH, which indicated that protons were involved in the electrode reaction. A linear relationship was observed between the oxidation peak potential and the solution pH with the linear regression equation as:

$$E_{pa}\,(V) = -0.037pH + 1.471 \quad (R^2 = 0.999)\ldots\ldots\ldots\ldots\ldots\ldots \text{(Eq. 2.3)}$$

The pulse amplitude has a profound effect upon the sensitivity of the adenosine response. Figure 2.20 shows the effect of different pulse amplitude, where the signal increases rapidly and nearly linearly between 5.0 and 50 mV, and then starts decreasing. All subsequent work thus employed 50 mV pulse amplitude.

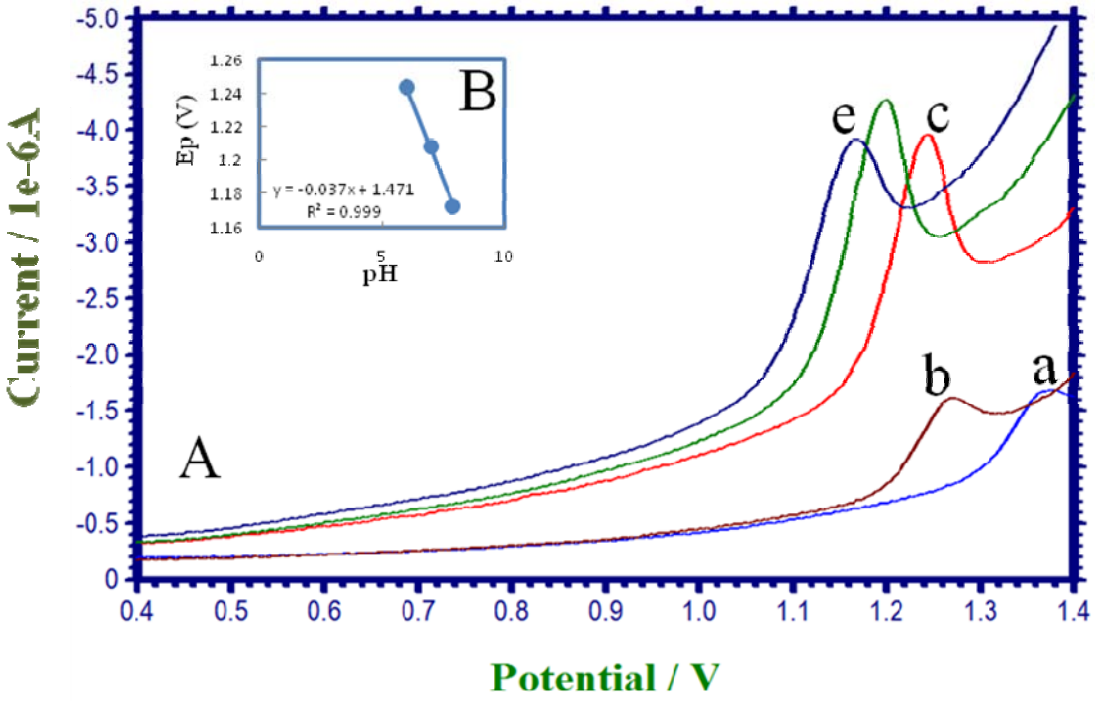


Figure 2.19. *(A) Square-wave stripping voltammograms of 5.0 ppm adenosine using GCPE in different buffer media with different pHs: 0.2 M acetate buffer, pH 4.0 (a), and pH 6.0 (b); 0.2 M phosphate buffer, pH 6.0 (c), pH 7.0 (d), and pH 7.9 (e). Electrode pretreatment, 1.0 min at +1.7 V; Accumulation time, 1.0 min at +0.5V. Potential step, 4 mV; Frequency, 25 Hz; Pulse amplitude, 50 mV. The corresponding plot.*

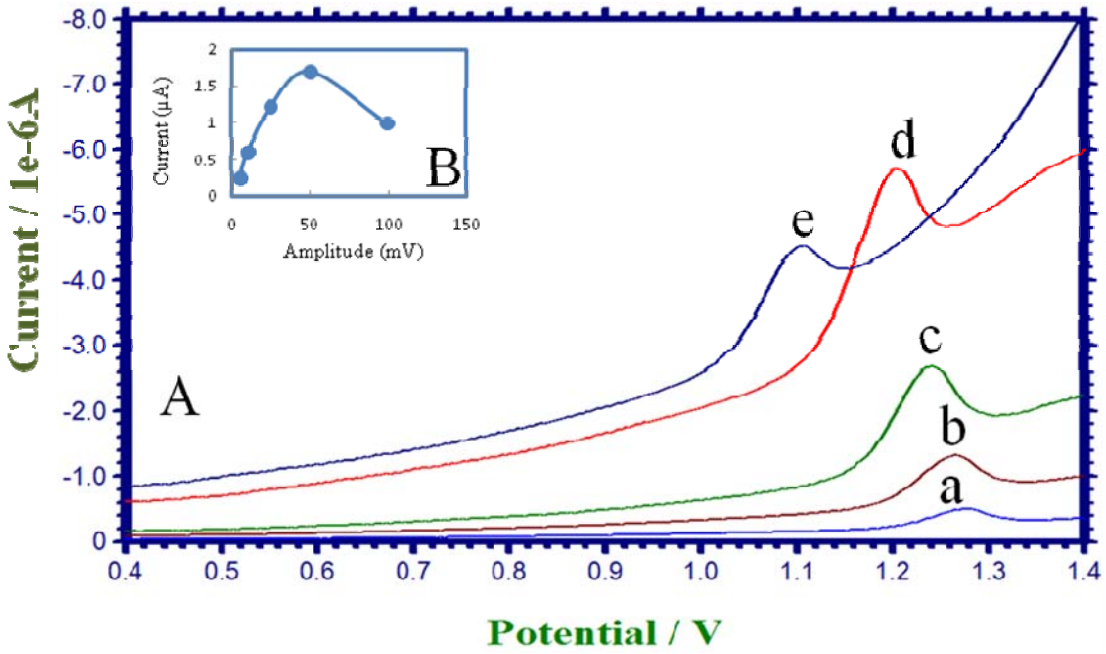


Figure 2.20. *(A) Square-wave stripping voltammograms of 5.0 ppm adenosine using GCPE at different pulse amplitude: 5.0 (a), 15 (b), 25 (c), 50 (d) and 100 (e) mV. Measurements were performed in phosphate buffer solution (0.2 M, pH7.0). (B) The corresponding plot. Other conditions, as in Figure 2.19.*

The effect of the frequency upon the adenosine signal is further examined in Figure 2.21A. The electrochemical responses of 5.0 ppm adenosine at frequencies 10, 25, 50, 100, and 200 Hz were obtained. It has been noticed that the larger the frequency, the higher the adenosine peak. Taking in consideration the noise generated at frequencies higher than 100 Hz, a frequency of 100 Hz was used on the subsequent experiments.

Another parameter affecting adenosine peak was optimized, (Figure 2.21B). The dependence of the stripping signal on the accumulation potential was examined over the -0.0 to +1.0 V range. The response rises gradually between 0.0 and +0.2 V, then decreases slowly and nearly levels off above +0.7 V. So for that +0.2 was chosen as an optimum detection potential.

The influence of accumulation time is studied as well. Figure 2.22 evaluates the effect of the accumulation time upon the stripping signal. The response rises with increasing the accumulation time up to 120 sec, and decreases slowly after that. The explanation of such behavior is that the surface of the electrode got saturated with adenosine molecules.

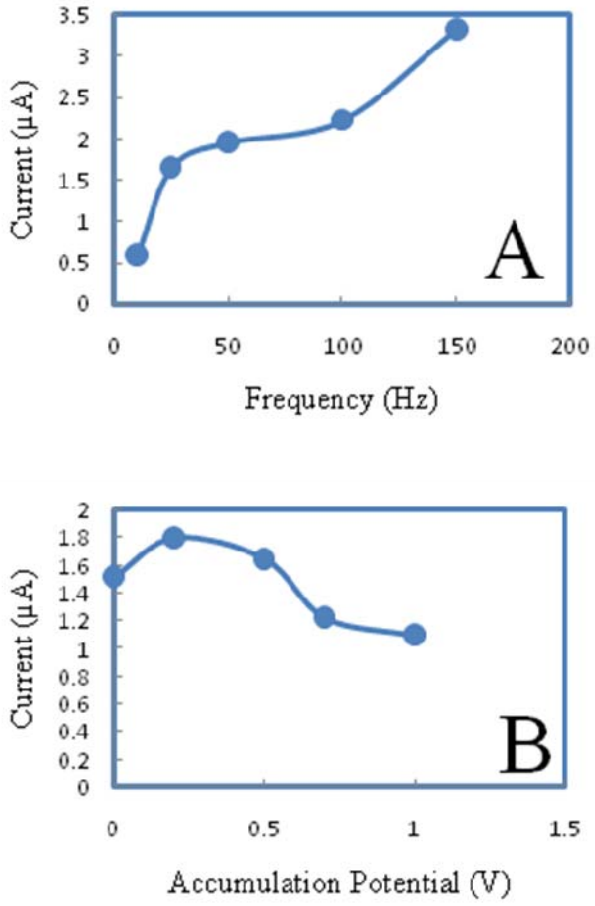


Figure 2.21. *Effect of frequency (A), and accumulation potential (B) upon the Square-wave stripping voltammetric response of 5.0 ppm adenosine using GCPE. Pulse amplitude, 50 mV. Other conditions, as in Figure 2.20.*

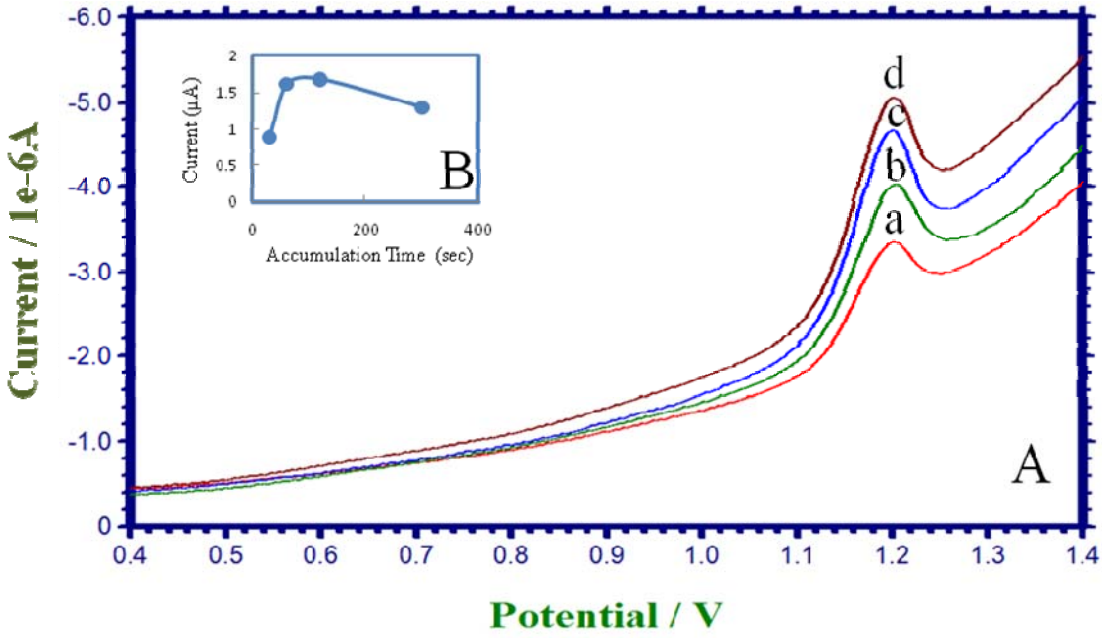


Figure 2.22. *(A) Square-wave stripping voltammograms of 5.0 ppm adenosine using GCPE at different accumulation times: 30 (a), 60 (b), 120 (c) and 300 (d) sec. (B) The corresponding plot. Other conditions, as in Figure 2.21.*

2.3.1.3.3. Analytical Determination

The quantitative behavior was assessed by monitoring the dependence of the adenosine peak height upon the concentration differences.

Typical calibration data are displayed in Figure 2.23. The response increases linearly with the target concentration up to 6.0 ppm, then more slowly, and levels off above 8.0 ppm. Analogous measurements of a 100 ppb adenosine solution were used to estimate the detection limit (not shown). A value of around 35 ppb was thus estimated based on the signal-to-noise characteristics of these data (S/N = 3). Such detection limit corresponds to 0.13 μmol/l. A series of seven repetitive measurements of 5.0 ppm adenosine solution was used for estimating the precision (not shown). This series yielded a highly relative standard deviation (RSD) of 0.1071.

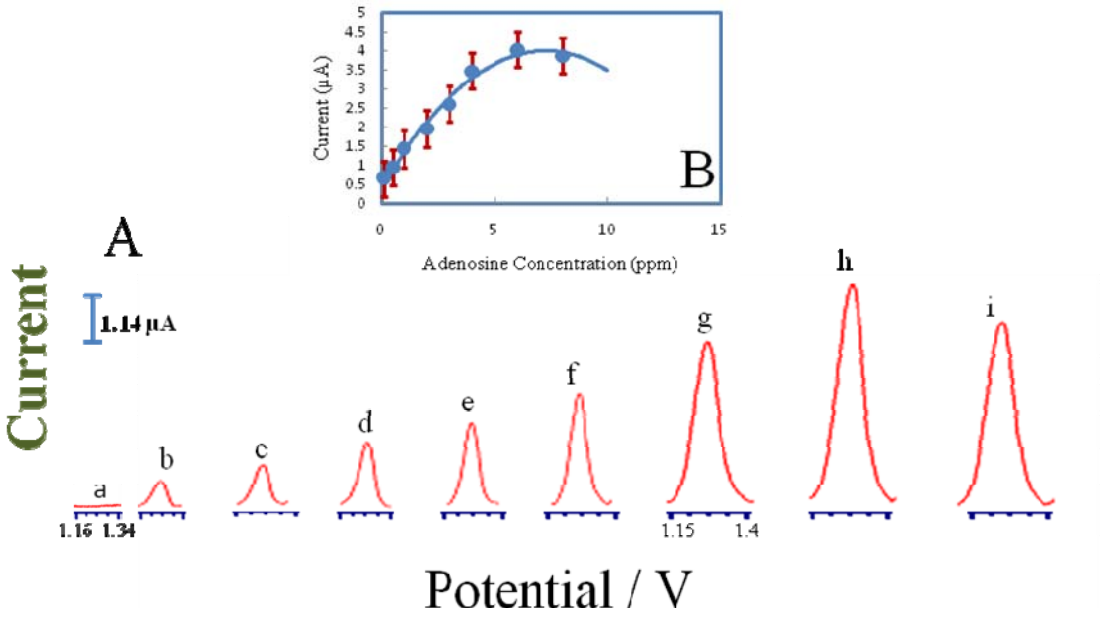


Figure 2.23. *(A) Square-wave stripping signals of adenosine at GCPE: 0.0 (a), 0.1 (b), 0.5 (c), 1.0 (d), 2.0 (e), 3.0 (f), 4.0 (g), 6.0 (h), and 8.0 (i) ppm. Measurements were performed in phosphate buffer solution (0.2 M, pH7.0). Electrode pretreatment, 1.0 min at +1.7 V; Accumulation time, 2.0 min at +0.2V. Potential step, 4 mV; Frequency, 100 Hz; Pulse amplitude, 50 mV. (B) The corresponding calibration plots.*

2.3.1.3.4. Interferences

In this section we report the effect of heavy metals such as (Cu, Cd, Pb), and some organic compounds on the obtained adenosine peak. As a representive of heavy metals, copper ions (Cu(II)) effect shown in Figure 2.26.

Figure 2.24 examines the comparison between square wave voltammograms at glassy carbon paste electrode for 5.0 ppm adenosine in absence (a) and in presence (b) of 2.0 ppm copper ions, Cu(II). A hiegher peak for adenosine was observed in presence of copper ions, with a very small shift in potential position.

We have studied in this section the interference effect of some organic compounds such as ascorbic acid, lactose, mannitol, EDTA and sodium dodecylbenzene sulfonate (SDBS) on the adenosine response. The selectivity for the measurement of adenosine was confirmed by measuring the responses to the addition of 1.0 ppm of the potential interferences, as shown in Table 2.3, Further study was obtained with adding different concentrations, with increasing the concentration of the interferences the precentage of signal changing increase.

2.3.1.3.5. Application

The developed detection method using the glassy carbon paste composite material was tested on the detection of adenosine in human urine, no clear peak appears with different concentrations of adenosine.

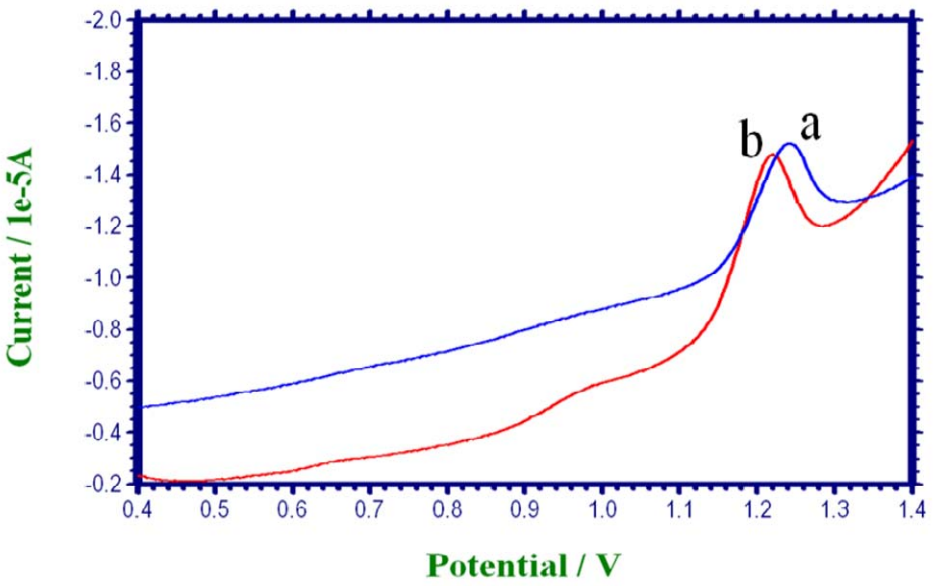


Figure 2.24. *Square-wave stripping voltammograms of 5.0 ppm adenosine in absence (a) and in presence (b) of 2.0 ppm Cu(II) using GCPE. Measurements were performed in phosphate buffer solution (0.2 M, pH7.0). Electrode pretreatment, 1.0 min at +1.7 V; Accumulation time, 2.0 min at -0.05V. Potential step, 4 mV; Frequency, 100 Hz; Pulse amplitude, 50 mV.*

Table 2.3. Influence of interferences on the determination of 5.0 ppm adenosine.

Interference	Concentration/ppm	Change of Signal (%)
Ascorbic Acid	1	3.9
	3	20.08
	5	21.8
Lactose	1	1.9
	3	12.2
	5	13.5
SDBS	1	3.4
	3	18.6
	5	54.6
Mannitol	1	10.0
	3	35.08
	5	41.6
EDTA	1	13.8
	3	20.7
	5	41.8

2.3.2. Using Glassy Carbon Electrode Surfaces

As mentioned above, we focus in this work on the use of different carbon materials as useful materials to be used for electroanalysis of different analytes.

2.3.2.1. Procedure

By using glassy carbon electrode, polishing with alumina powder and rinsing with deionized water should be done for the electrode surface before each measurement. After accumulation at +0.2 V in a stirred solution of 0.2 M phosphate buffer (pH7.0) containing a specific concentration of adenosine. The stirring was then stopped for 5s; this was followed by a subsequent stripping using a square wave voltammetric waveform, with a 4.0 mV potential step, 25Hz frequency and amplitude of 50 mV.

2.3.2.2. Results and Discussion

2.3.2.2.1. Electrochemical Investigation

Figure 2.25 shows one irreversible oxidation peak for 5.0 ppm adenosine at the glassy carbon electrode surface.

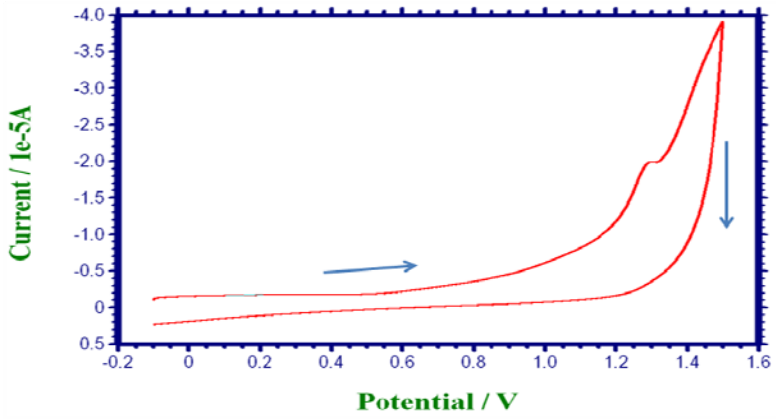


Figure 2.25. *Cyclic voltammogram of 5.0 ppm adenosine using GCE at scan rate 100 mV/s. Measurements were performed in phosphate buffer solution (0.2 M, pH7.0). Accumulation time 30 sec at +0.2V.*

2.3.2.2.2. Optimization

Optimization of the parameters of the electrochemical technique, square-wave voltammetry, used in the current study was done as shown in Figures 2.26-2.28.

Figure 2.26A, shows typical square-wave stripping voltammograms of 5.0 ppm adenosine using GCE at different pulse amplitude, the measurements were performed in phosphate buffer solution (0.2 M, pH7.0). From the obtained voltammograms, and corresponding plot, Figure 2.26B, the height of the adenosine peak increases with increasing the amplitude up to 50 mV then decreases thereafter. All subsequent work thus employed 50 mV pulse amplitude.

In contrast, effect of frequency as well as accumulation time effect were studied in the following Figures 2.27 and 2.28 respectively. Higher response was obtained with applying higher frequency but with respecting to the best shape frequency 25 Hz was chosen to be the optimum parameter (Figure 2.27B).

Thirty second accumulation time was the best as can be noticed from the Figure 2.28, the peak current start decreasing with using more accumulation times. Such profile could be attributed to the surface saturation of glassy carbon paste with adenosine molecules. So 30 sec accumulation time was chosen.

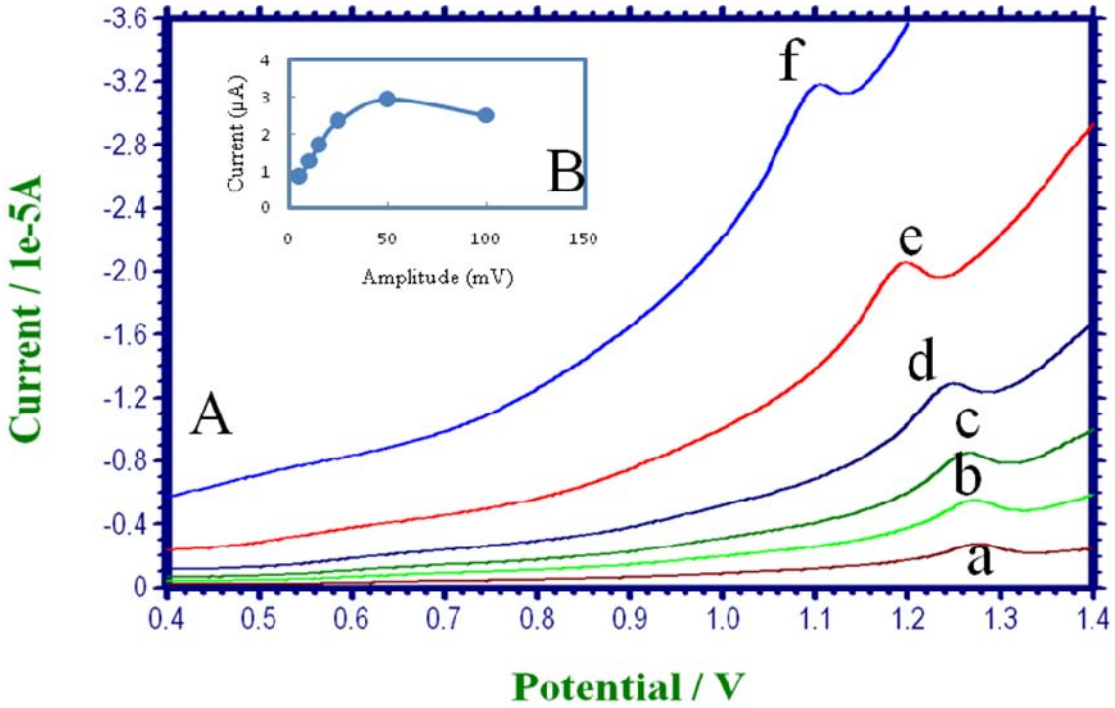


Figure 2.26. *(A) Square-wave stripping voltammograms of 5.0 ppm adenosine using GCE at different pulse amplitude: 5.0 (a), 10 (b), 15 (c), 25 (d), 50 (e) and 100 (f) mV. Measurements were performed in phosphate buffer solution (0.2 M, pH7.0). Accumulation time, 30 sec at +0.5V. Potential step, 4 mV; Frequency, 25 Hz; (B) The corresponding plot.*

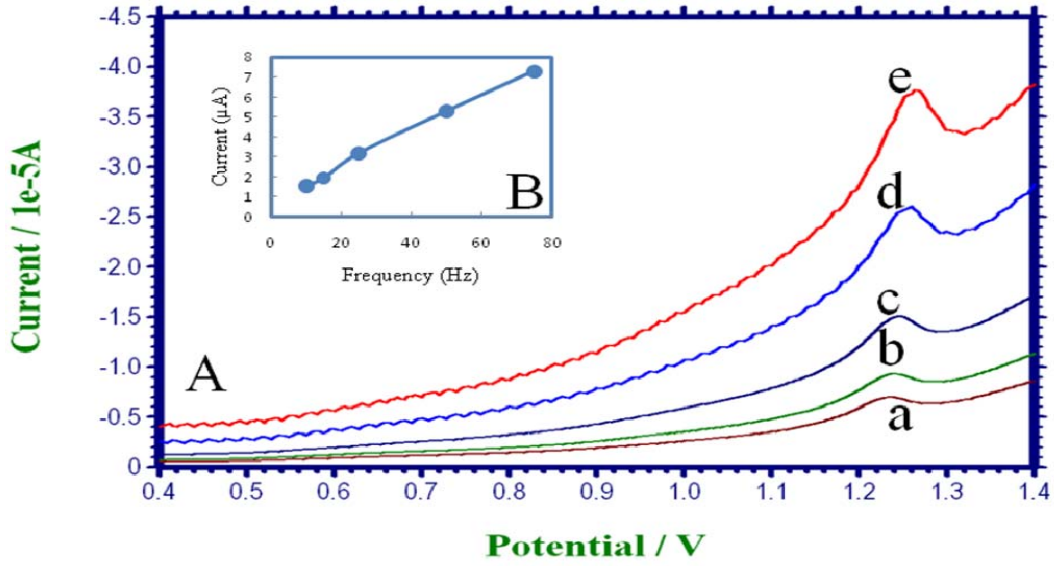


Figure 2.27. *(A) Square-wave stripping voltammograms of 5.0 ppm adenosine using GCE at different frequency: 5.0 (a), 15 (b), 25 (c), 50 (d), and 75 (e) mV. (B) The corresponding plot. Other conditions, as in Figure 2.26.*

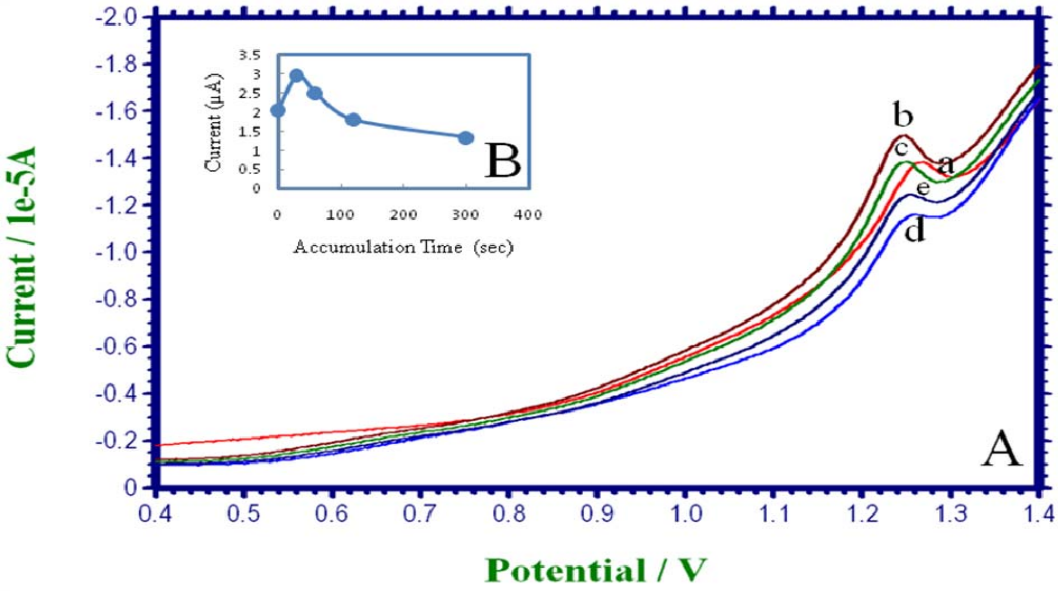


Figure 2.28. *(A) Square-wave stripping voltammograms of 5.0 ppm adenosine using GCE at different accumulation times: 0.0 (a), 30 (b), 60 (c), 120 (d) and 300 (e) sec. (B) The corresponding plot. Other conditions, as in Figure 2.26.*

2.3.2.2.3. Analytical Determination

Quantitative evaluation is based on the dependence of the peak height on the concentration of the adenosine. Figure 2.29A displays the square wave stripping voltammetric responses obtained at different adenosine concentrations using glassy carbon electrode (GCE). The response increases linearly with the target concentration up to 12 ppm, and levels off at higher concentrations. The corresponding plots supporting these results, (Figure 2.29B).

Relative standard deviation was calculated after seven measurements for 5.0 ppm adenosine, (Figure 2.30). The result is RSD of 0.3141.

A well defined peak was obtained (not shown) for a concentration of adenosine as low as 1.0 ppm, and a detection limit of around 300 ppb (31.25 μmol/l) was estimated according to Signal/Noise ratio equal to three.

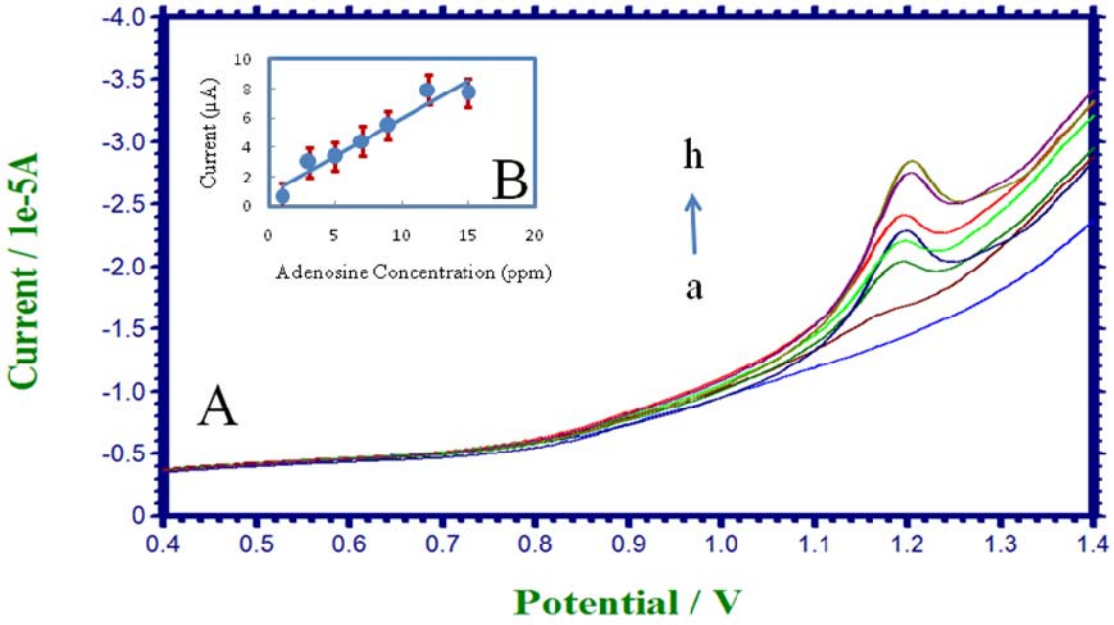


Figure 2.29. *(A) Square-wave stripping voltammograms of adenosine concentrations using GCE: 0.0 (a), 1.0 (b), 3.0 (c), 5.0 (d), 7.0 (e), 9.0 (f), 12 (g) and 15 (h) ppm. Measurements were performed in phosphate buffer solution (0.2 M, pH7.0). Accumulation time, 30 sec at +0.2V. Potential step, 4.0 mV; Frequency,25 Hz; Pulse amplitude, 50 mV. (B) The corresponding calibration plot.*

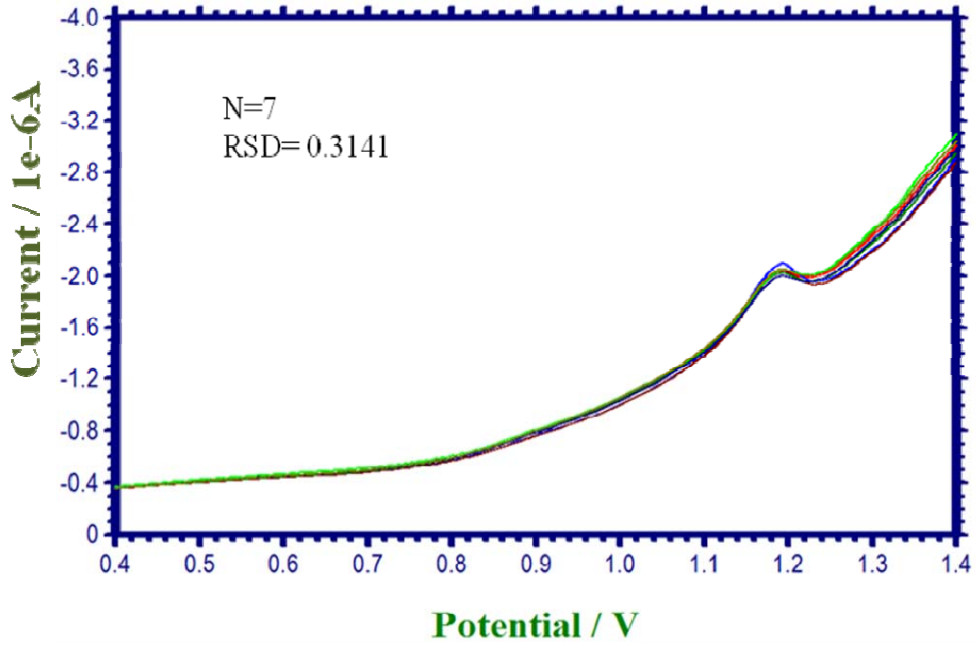


Figure 2.30. *Square-wave stripping voltammograms of 5.0 ppm adenosine using GCE. a series of measurements of seven different adenosine samples and at a new surface every time. Other conditions, as in Figure 2.*29

2.3.3. Using Graphite Pencil Electrode Surfaces

2.3.3.1. Procedure

The pencil was fixed vertically with 11 mm of the pencil extrude outside and 6.0 mm of the lead that will immerse in the solution. A short 30s electrochemical pre-treatment at +1.4V (using the blank phosphate buffer solution; 0.2 M, pH 7.0) preceded the measuring step 60s accumulation at +0.2 V in a stirred solution of phosphate buffer (0.2 M, pH7.0) containing a specific concentration of adenosine. The stirring was then stopped for 5s; this was followed by a subsequent stripping using a square wave voltammetric waveform, with a 4.0 mV potential step, 25Hz frequency and amplitude of 50 mV.

2.3.3.2. Results and Discussion

2.3.3.2.1. Analytical Determination

By using another carbon electrode, graphite pencil electrode (GPE), the calibration study obtained showed an increase of the adenosine peak with the increase of the adenosine concentration. As can be shown in Figure 2.31.

Using graphite pencil electrode (GPE), the detection limit is 166.6 ppb (0.62 μmol/l) which is better than that obtained for glassy carbon electrode (1.25 μmol/l), however it is less than the detection limit obtained using glassy carbon paste electrode (0.13 μmol/l). On the other hand, a series of seven measurements of 5.0 ppm adenosine gives a highly reproducible response and the best among other electrodes with a relative standard deviation 0.0777.

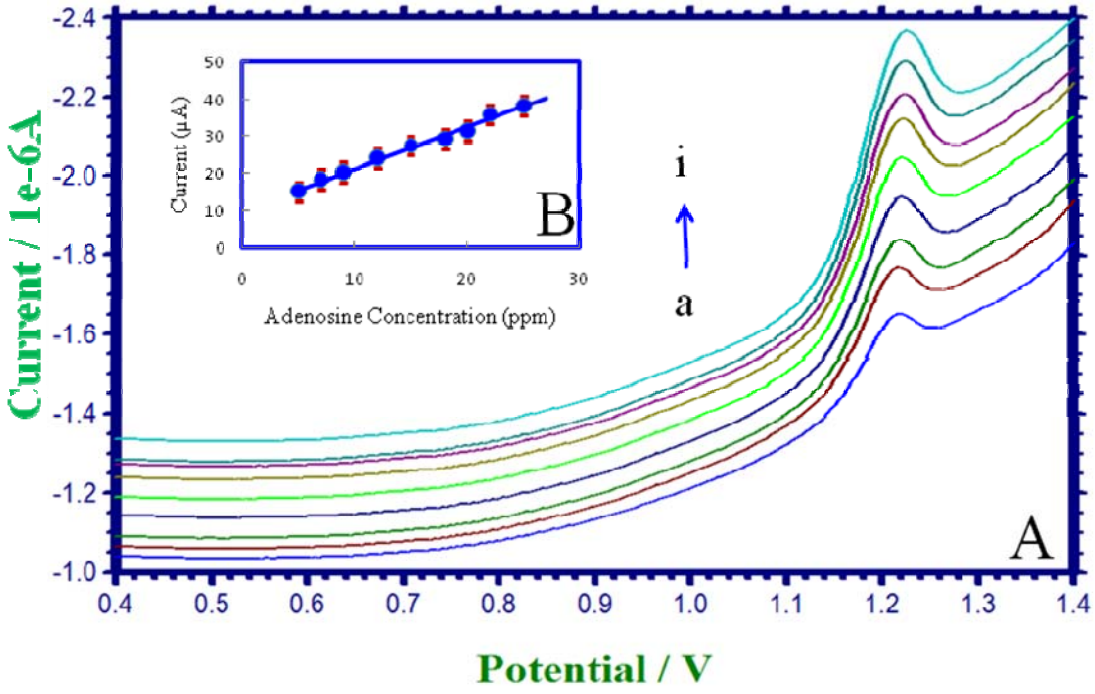


Figure 2.31. *(A) Square-wave stripping voltammograms of adenosine concentrations using GPE: 5.0 (a), 7.0 (b), 9.0 (c), 12 (d), 15 (e), 18 (f), 20 (g), 22 (h), and 25 (i) ppm. Measurements were performed in phosphate buffer solution (0.2 M, pH7.0). Electrode pretreatment, 30 sec at +1.4 V; Accumulation time, 1.0 min at +0.2V. Potential step, 4 mV; Frequency, 25 Hz; Pulse amplitude, 50 mV. (B) The corresponding calibration plot.*

Table 2.4 summarizes the results related to detection limits and the relative standard deviations (RSD) for adenosine at the different electrodes (GCPE, GCE, and GPE).

A series of seven measurements of 5.0 ppm adenosine at each electrode surfaces were measured to get the RSD. As we can notice, graphite pencil electrode is the best one related to the relative standard deviation of 0.0777.

Glassy carbon paste electrode (GCPE) gives the lowest detection limit (0.13 μmol/l) estimated based on the signal-to-noise characteristics of these data (S/N = 3). Such detection limit corresponding to a calculated value of around 35.0 ppb.

Table 2.4. The detection limits and relative standard deviations for adenosine at glassy carbon paste (GCPE), glassy carbon (GCE), and graphite pencil (GPE) electrodes.

Electrode	**Detection Limit (µmol/l)**	**Relative Standard Diviation**
GCPE	0.13	0.1071
GCE	1.25	0.3141
GPE	0.62	0.0777

CHAPTER 3

3. Electrochemical Investigation and Analytical Determination of Guanine and Guanosine

3.1. Introduction

The last decades have seen the emergence of a tremendous interest for DNA detection for medical, pharmaceutical or environmental applications. Several techniques have been proposed, such as capillary zone electrophoresis (CZE) (73-75) and electrochemiluminescence (76). A few studies on the electrochemical oxidation of the nucleic acid constituents were done as well (77-79).

In the present work, the oxidation behavior of guanine and its nucleoside, guanosine, are investigated by different voltammetric techniques at different carbon electrode surfaces.

3.2. Electrochemical Investigation and Analytical Determination of Guanine

3.2.1.Using Glassy Carbon Paste Electrode Surfaces

3.2.1.1. Apparatus

Voltammetry measurements were performed with an electrochemical work station (CHI660C, CH Instruments Inc, Austin, TX, USA). The Ag/AgCl reference electrode (in 3M KCl, CHI111, CH Instruments Inc), and platinum wire counter electrode (CHI115, CH Instruments Inc) were inserted into the 1.0 ml glass cell through holes in its Teflon cover.

3.2.1.2. Electrode Preparation

The glassy carbon Paste electrode (GCPE) was prepared by hand-mixing 70 mg of 20-50 μm glassy carbon powder with 30 mg of mineral oil. The portion of the resulting paste was then packed firmly into the electrode cavity (1.0 mm diameter and 2.0 mm depth) of the PTFE sleeve. Electrical contact was established via a copper wire. The paste surface was smoothed with a weighing paper, and then rinsed carefully with deionized water prior to each measurement.

3.2.1.3. Reagents

Guanine, sodium acetate and phosphate buffer solutions with different pHs, that were served as the supporting electrolyte, were obtained from Sigma. Glassy carbon spherical powders (20 – 50 μm) were obtained from Alfa Aesar (Ward Hill, MA, USA). The mineral oil, copper atomic absorption standard solutions (containing 1000 μg/ml Cu, with 0.5 mol/l HNO_3), cadmium atomic absorption standard solutions (containing 1000 μg/ml Cd(II), with 0.5 mol/l HNO_3), lead atomic absorption standard solutions (containing 1000 μg/ml Pb(II), with 0.5 mol/l HNO_3), were purchased from Aldrich. Mannitol and EDTA was obtained from BDH Analar (Poole, England), lactose was purchased from B.B.L (Baltimore, Maryland, USA), sodium dodecyl benzene sulfonate was purchased from Science lab. Com (Houston, Texas, USA), while ascorbic acid was obtained from Fluka AG (Buchs, Switzerland). All stock solutions were prepared using deionized water

3.2.1.4. Procedure

In order to obtain reproducible results, a standard pretreatment procedure was applied before recording each measurement.

Cyclic voltammetry (CV) measurements were performed by treating the surface of the electrode at +1.7 V for 60s followed by 120s accumulation at +0.5 V in a stirred solution of phosphate buffer (0.2 M, pH6.0) (or as mentioned otherwise) containing a specific concentration of guanine. The stirring was stopped for 5s; before scanning the potential between -0.2 to +1.6 V at scan rate of 100 mV/s.

The square wave voltammetry (SWV) measurements were performed by treating the surface of the electrode at +1.7 V for 60s followed by 120s accumulation at +0.5 V in a stirred solution of 0.2 M phosphate buffer (pH6.0) containing a specific concentration of guanine. The stirring was stopped for 5s; and then followed by a subsequent stripping using a square wave voltammetric waveform, with a 4.0 mV potential step, 100 Hz frequency and amplitude of 50 mV(or as mentioned otherwise). A new glassy carbon paste surface was used in every measurement. The electrode surface was smoothed and rinsed carefully with deionized water prior each measurement.

3.2.1.5. Results and Discussion

3.2.1.5.1. Electrochemical Investigation

The initial study involved the cyclic voltammetric behavior of guanine base. Figure 3.1 depicts the cyclic voltammogram of guanine in phosphate buffer (0.2 M, pH6.0) at glassy carbon paste electrode surface. As can be seen, one oxidation peak with an irreversible process happened at position +0.81 V (vs. Ag.AgCl/KCl)

In order to study the adsorption of the analyte on the surface of the electrode, the effect of the scan rate (U) on the peak current of this compound was examined, (Figure 3.2). The electrochemical oxidation peak of the guanine increased markedly with the increase in the scan rate (U) up to 150 mV/s and then levels off. This behavior is typical of an electrode process where the reactant is adsorbed on the electrode. Figure 3.2B, shows these results.

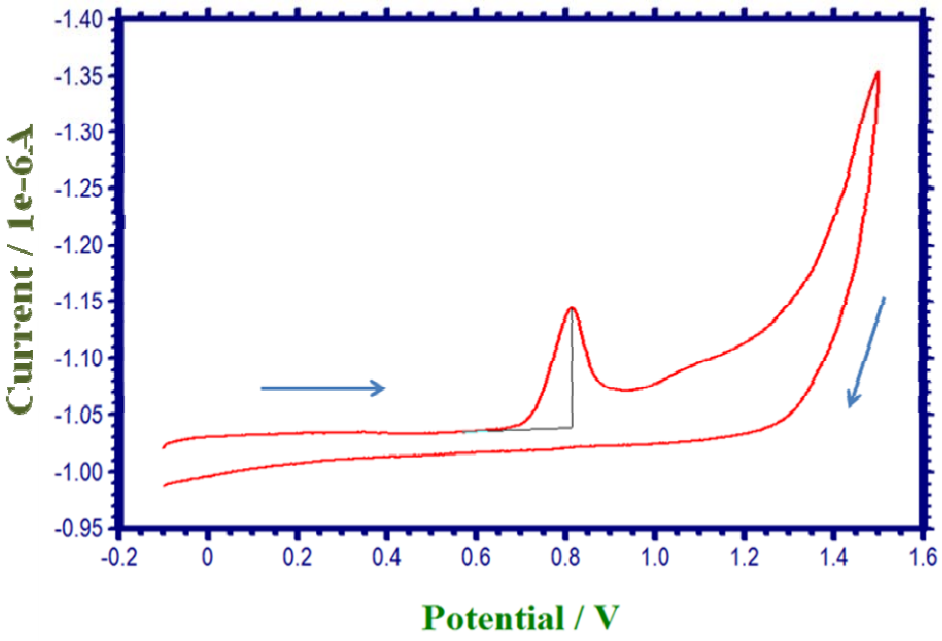


Figure 3.1. *Cyclic voltammogram of 1.0 ppm guanine using GCPE at scan rate 100 mV/s. Measurements were performed in phosphate buffer solution (0.2 M, pH6.0). Electrode pretreatment 1.0 min at +1.7 V. Accumulation time 2.0 min at +0.5V.*

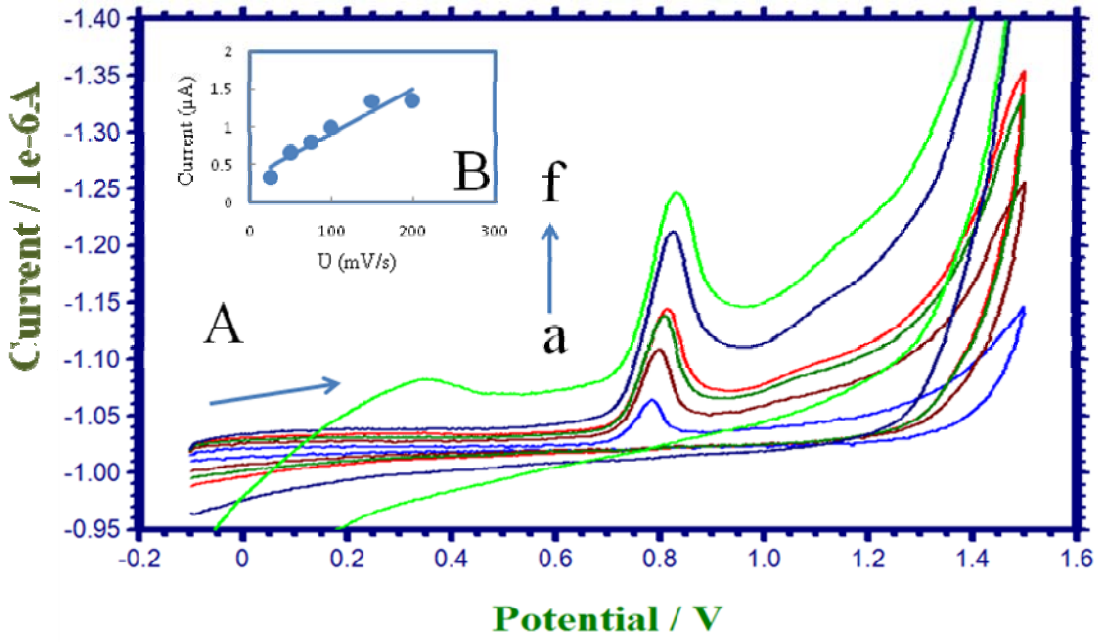


Figure 3.2. *(A) Cyclic voltammograms of 1.0 ppm guanine using GCPE with different scan rates, 25 (a), 50 (b), 75 (c), 100 (d), 150 (e), and 200 (f) mV/s. (B) The corresponding plots. Other conditions, as in Figure 3.1.*

3.2.1.5.2. Optimization

The quality of the voltammetric analysis depends on a number of parameters, in particular on the type of buffer and its pH, pulse amplitude, frequency, accumulation potential and accumulation time.

Figure 3.3 displays the influence of different buffer with different pHs, this Figure 3.3 shows the square-wave stripping voltammetric responses of 5.0 ppm guanine at newly prepared glassy carbon paste electrode (GCPE) surfaces. The peaks in phosphate buffer are higher than that in acetate buffer. From Figure 3.3 (c, d, e) one can conclude that the best guanine response with respect to peak potential, height and shape, was obtained in phosphate buffer solution of pH 6. As to adenine, similar studies were also performed to confirm that protons were involved in the electrode reaction. A good linear relationship was established between the oxidation peak potential and the solution pH with the linear regression equation (Eq. 3.1):

$$E_{pa}\,(V) = -0.065pH + 1.141 \qquad (R^2 = 0.992)\ldots\ldots\ldots\ldots\ldots\ldots \text{ (Eq. 3.1)}$$

The optimization results of three different parameters are represented in Figure 3.4. As the pulse amplitude increases (Figure 3.4A), the response increases rapidly up to 50 mV then starts decreasing thereafter, so 50 mV was chosen as an optimum. In Figure 3.4 B, the effect of frequency was studied, the larger the frequency, the higher the peak. Yet with consideration of the fact that the noise starts appearing at frequencies above 100 Hz. Thus, frequency of 100 Hz was chosen on the subsequent experiments. As we have seen

in the previous chapter, short electrochemical activation is essential for trace measurements of nucleic acid constituents for obtaining better stripping signals. For that reason, accumulation potential was optimized within the range 0.0 to +1.0 V and plotted in Figure 3.4C. The value of +0.5 V was chosen as an optimum accumulation potential, where a maximum signal was obtained.

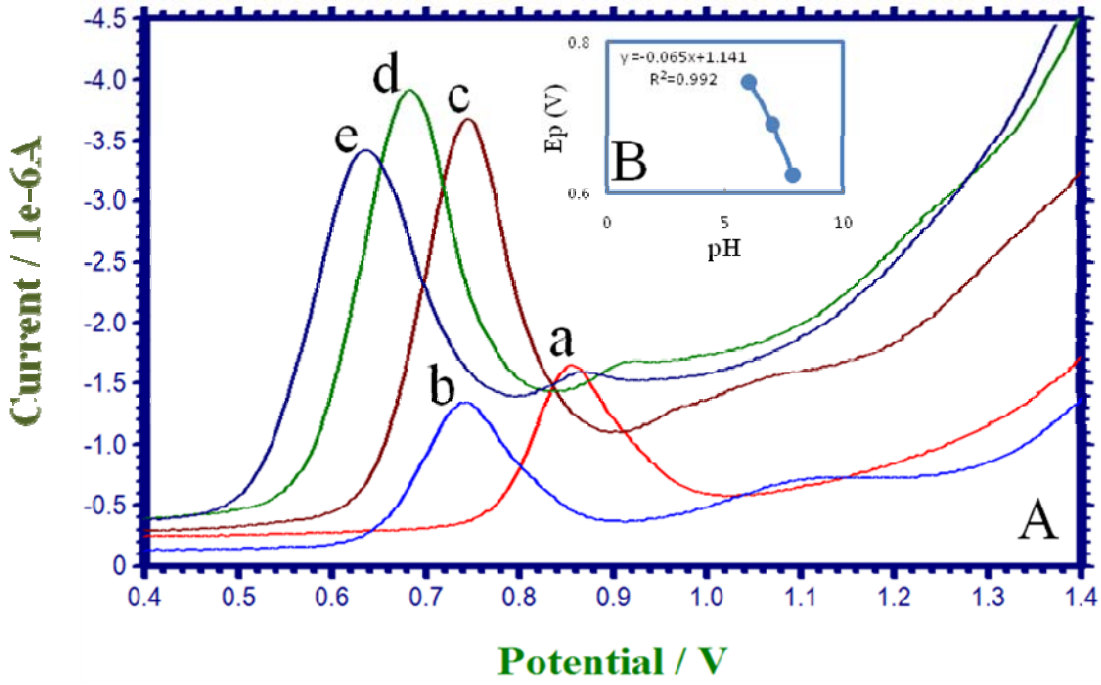


Figure 3.3. *Square-wave stripping voltammograms of 5.0 ppm guanine using GCPE in different buffer media with different pHs: 0.2 M acetate buffer, pH 4.0 (a), pH 6.0 (b); 0.2 phosphate buffer, pH 6.0 (c), pH 7.0 (d), and pH 7.9 (e). Electrode pretreatment, 1.0 min at +1.7 V; Accumulation time, 1.0 min at +0.5V. Potential step, 4 mV; Frequency, 25 Hz; Pulse amplitude, 50 mV.*

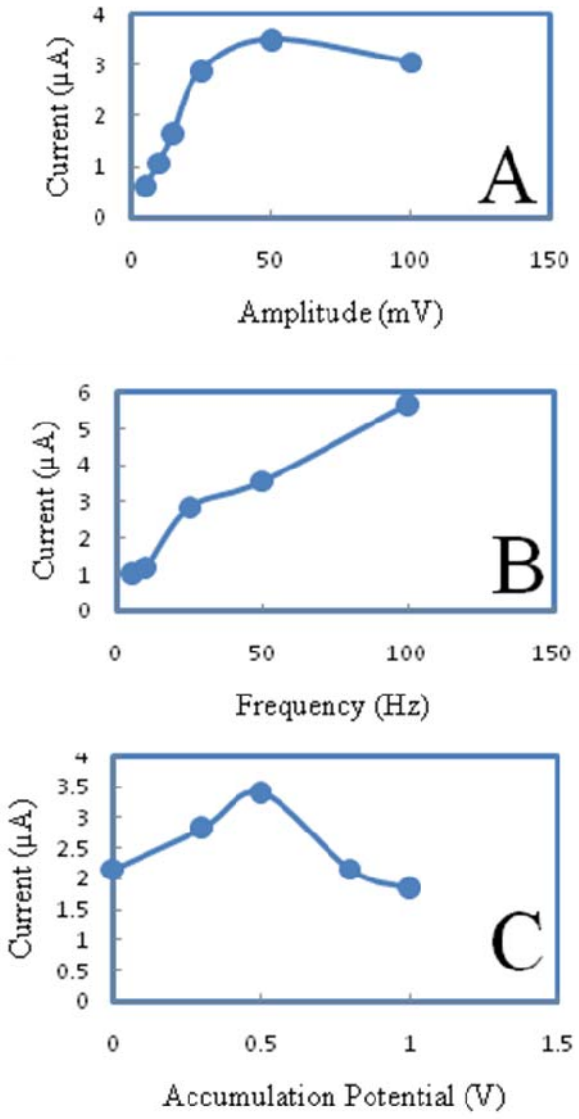


Figure 3.4. *Effect of pulse amplitude (A), frequency (B), and accumulation potential (C) upon the square-wave stripping voltammetric response of 5.0 ppm guanine using GCPE. Measurements were performed in phosphate buffer solution (0.2 M, pH6.0). Electrode pretreatment, 1.0 min at +1.7 V. Accumulation time, 1.0 min.*

The effect of the accumulation time upon guanine electrochemical signal is further examined in Figure 3.5. The peak height increases in a nearly linear fashion up to 120s and then starts decreasing. Such profile due to the total coverage of the electrode surfaces with guanine molecules. The corresponding plot supports this result, (Figure 3.5B). All subsequent work thus employed a 120s as an accumulation time.

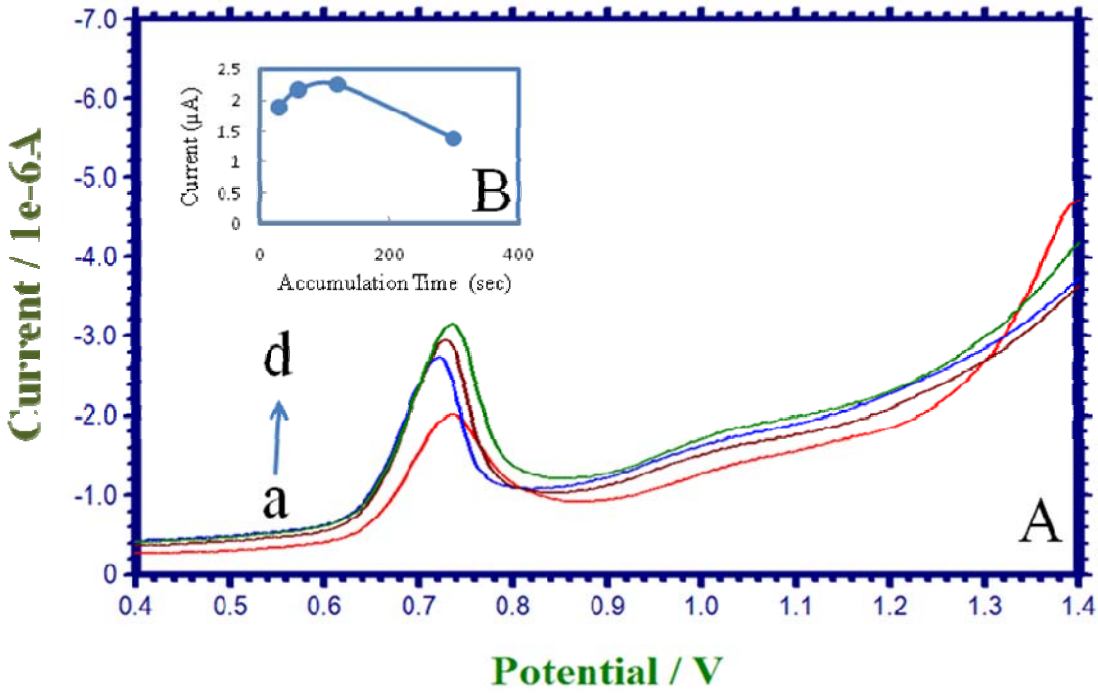


Figure 3.5. *(A) Square-wave stripping voltammograms of 1.0 ppm guanine using GCPE at different accumulation times: 30(a), 60(b), 120(c) and 300(d) sec. Measurements were performed in phosphate buffer solution (0.2 M, pH6.0). (B) The corresponding plot. Other conditions, as in Figure 3.3.*

3.2.1.5.3. Analytical Determination

The calibration curve is charactarized by a correlation between the guanine concentration and the corresponding peak current.

Under the optimum conditions, the square wave stripping voltammetric responses were obtained at different guanine concentrations as shown in Figure 3.6. With increasing the guanine concentration within the range of 0.1 – 8.0 ppm, the peak current increases nearly linearly. Further increase in the concentration causes a marked non-linear influence on the peak current.

The limit of detection, defined as the sample concentration that produces a peak with a height three times the level of the baseline noise, a well defined peak was obtained (not shown) for a concentration of guanine as low as 50 ppb. A detection limit of 16.6 ppb (0.11 μmol/l) was estimated. The reproducibility of the guanine response at the GCPE was tested, with a relative standard deviation (RSD) of 0.1680, caleculated after a sereis of seven measurements of 1.0 ppm guanine.

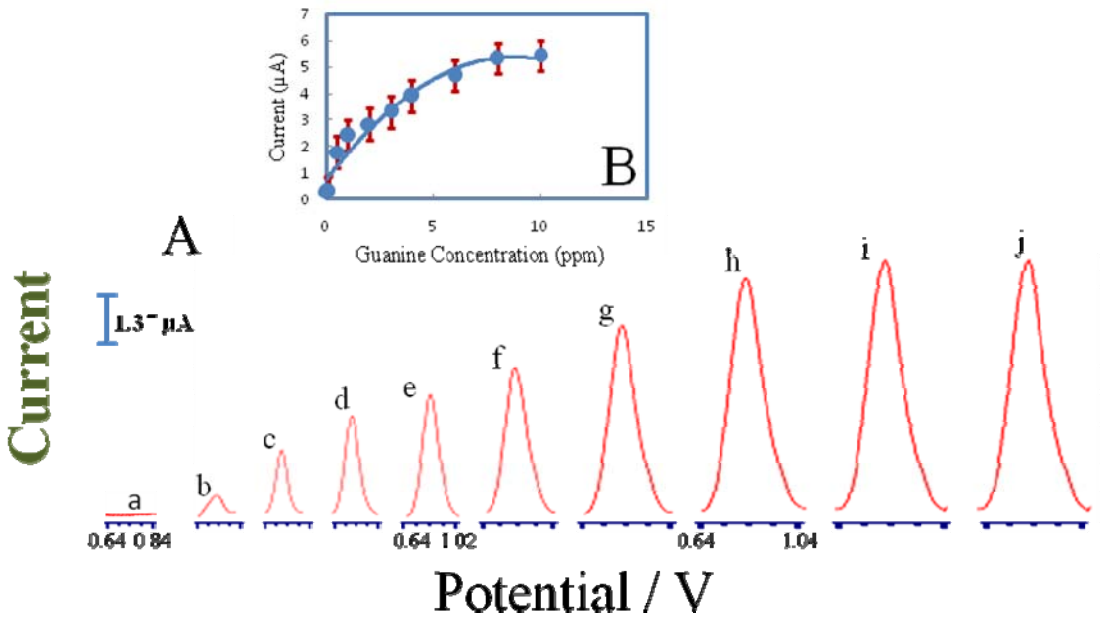


Figure 3.6. *(A) Square-wave stripping signals of guanine at GCPE: 0.0 (a), 0.1 (b), 0.5 (c), 1.0 (d), 2.0 (e), 3.0 (f), 4.0 (g), 6.0 (h), 8.0 (i), and 10 (j) ppm. Measurements were performed in phosphate buffer solution (0.2 M, pH6.0). Electrode pretreatment, 1.0 min at +1.7 V; Accumulation time, 2.0 min at +0.5V. Potential step, 4 mV; Frequency, 100 Hz; Pulse amplitude, 50 mV. (B) The corresponding calibration plots.*

3.2.1.5.4. Interferences

As another nucleic acid constituent, guanine response was influenced by metal ions and few organic compounds. Some of these interferences increase the guanine signal, others decrease it.

In this section, we focus mainly on the effect of copper ions (Cu(II)) from all other metals we have tested which are (Pb, Cd), and some organic compounds on the response of the guanine moiety.

Figure 3.7 reflects the increasing in the 1.0 ppm guanine peak after 2.0 ppm of copper ions, Cu(II) is added (Figure 3.7b). Also a small shift to higher potential was observed for the guanine peak.

The method selectivity was studied using different interferences for example, ascorbic acid, lactose, mannitol, EDTA and SDBS. As a result, no interference at concentration 1.0 ppm of mannitol was observed. While, small changes in the *Ip* were observed with other compounds. With increasing the interferences concentration more decreases in the signal are observed (Table 3.1)

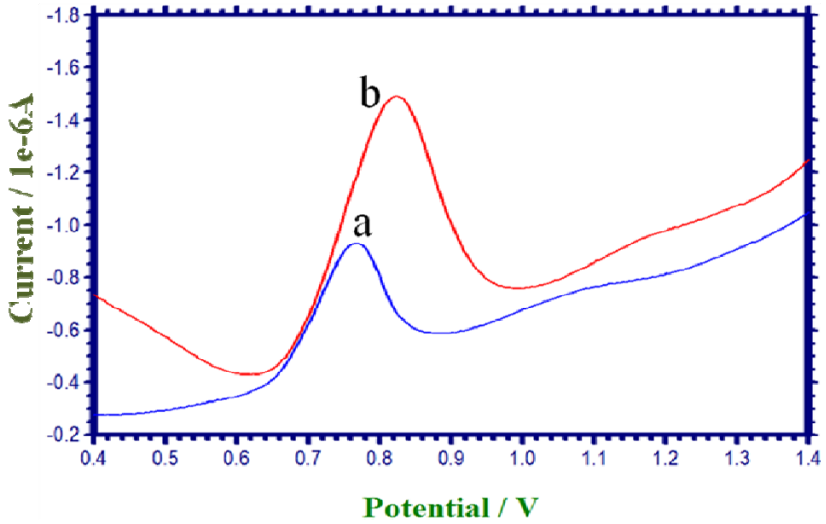


Figure 3.7. *Square-wave stripping voltammograms of 1.0 ppm guanine in absence (a) and presence (b) of 2.0 ppm Cu(II) using GCPE. Measurements were performed in phosphate buffer solution (0.2 M, pH6.0). Electrode pretreatment, 1.0 min at +1.7 V; Accumulation time, 2.0 min at -0.05V. Potential step, 4 mV; Frequency, 100 Hz; Pulse amplitude, 50 mV.*

Table 3.1. Influence of interferences on the determination of 1.0 ppm guanine.

Interference	Concentration/ppm	Change of Signal (%)
Ascorbic Acid	1	3.8
	3	12.8
	5	19.3
Lactose	1	14.2
	3	27.4
	5	36.9
SDBS	1	17.9
	3	23.4
	5	42.9
Mannitol	1	0.0
	3	15.5
	5	24.8
EDTA	1	2.26
	3	2.4
	5	5.2

3.2.1.5.5. Application

As low as micromolar detection limit is well suitable for the analysis of the guanine in human urine. The developed method for guanine detection at the glassy carbon paste composite material was examined on the detection of guanine in human urine medium. The human urine sample was diluted with deionized water (by volume 1:10). In a micro centrifuge tube, 10 µl of 100 ppm of guanine solution was added to 1.0 ml of the diluted urine. The final guanine concentration added to the sample was 1.0 ppm. Figure 3.8 shows a very clear peak for 1.0 ppm guanine in urine sample (Figure 3.8b) compare to the blank one (Figure 3.8a). The obtained results support the possibility of detecting nucleic acid constituent, eg. guanine, in biological media. eg. (human urine)

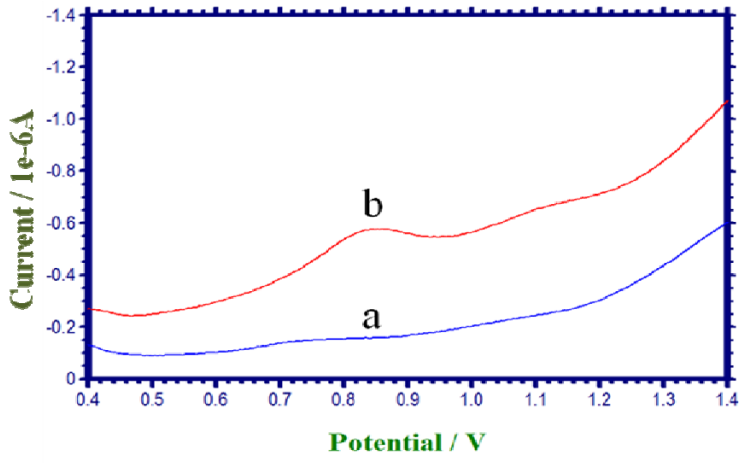


Figure 3.8. *Square-wave stripping voltammogram of 1.0 ppm guanine using GCPE in Urine medium. Other conditions, as in Figure 3.8.*

3.2.2. Using Glassy Carbon Electrode Surfaces

3.2.2.1. Electrode Preparation

The glassy carbon electrode (GCE) 3.0 mm diameter was polished with 0.3 μm alpha alumina powder and rinsed with deionized water before each measurement.

3.2.2.2. Procedure

Using glassy carbon electrode, polishing with alumina powder and rinsing with deionized water was done before each measurement. A 30s accumulation at +0.5 V was completed in a stirred solution of phosphate buffer (0.2 M, pH6) containing a specific concentration of guanine, the stirring was then stopped for 5s; and followed by a subsequent stripping using a square wave voltammetric waveform, with a 4.0 mV potential step, 25 Hz frequency and an amplitude of 50 mV.

3.2.2.3. Results and Discussion

3.2.2.3.1. Electrochemical Investigation

Guanine sample 5.0 ppm was electrochemically investigated on the glassy carbon electrode surfaces. Same as other bases, one oxidation peak with an irreversible process appeared when cyclic voltammetry was used. In Figure 3.9 the corresponding cyclic voltammogram is shown.

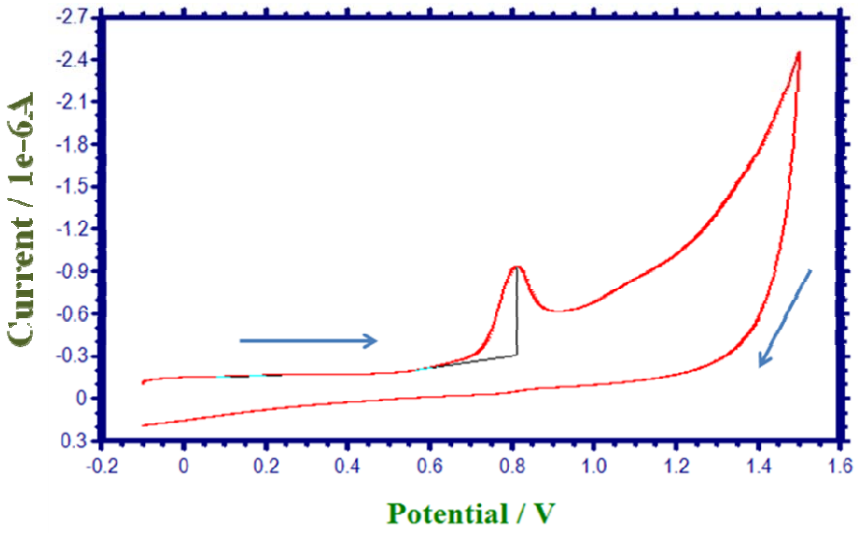


Figure 3.9. *Cyclic voltammogram of 5.0 ppm guanine using GCE at scan rate 100 mV/s. Measurements were performed in phosphate buffer solution (0.2 M, pH6.0). Accumulation time 30 sec at +0.5V.*

3.2.2.3.2. Optimization

In this part we could find the optimum conditions for the guanine base using square wave voltammetric technique at GCE surface.

First we started with studying the affect of pulse amplitude. As we can see in Figure 3.10, the guanine oxidation peak increases linearly with increasing the amplitude up to 50 mV then starts to decrease slowly. 50 mV pulse amplitude was chosen as an optimum.

Figure 3.11A shows the effect of frequency on the guanine response, as expected the larger the frequency, the higher the peak current. However the shape of the guanine peak has become noisy at a frequency higher than 25 Hz. So 25 Hz frequency is employed on all subsequent experimental work.

The dependence of the stripping voltammetric peaks height upon the accumulation time is shown in Figure 3.11B, the peak rises very sharply when 30s accumulation time applied then levels off till 120s to start decreasing gradually thereafter. Apparently, such a period of 30s is sufficient for a complete coverage of the electrode surface with the analyte molecules. Similar period (30s) was employed in the subsequent work.

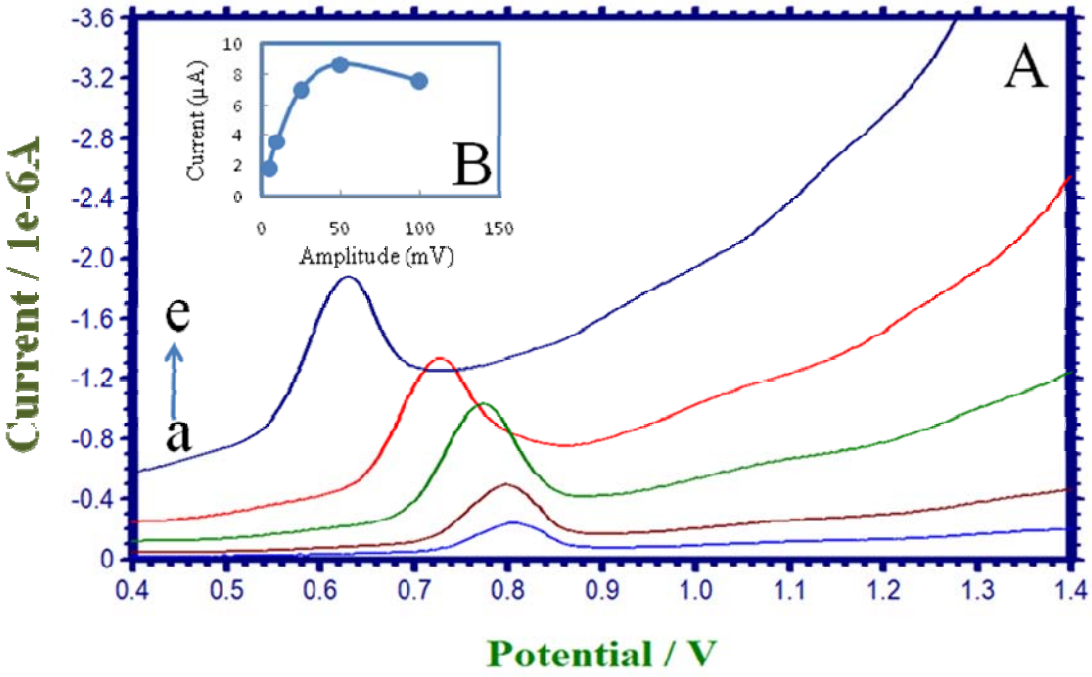


Figure 3.10. *(A) Square-wave stripping voltammograms of 5.0 ppm guanine using GCE at different pulse amplitudes: 5.0 (a), 15 (b), 25 (c), 50 (d) and 100 (e) mV. Measurements were performed in phosphate buffer solution (0.2 M, pH6.0). Accumulation time, 30 sec at +0.5V. Potential step, 4 mV; Frequency, 25 Hz; (B) The corresponding plot.*

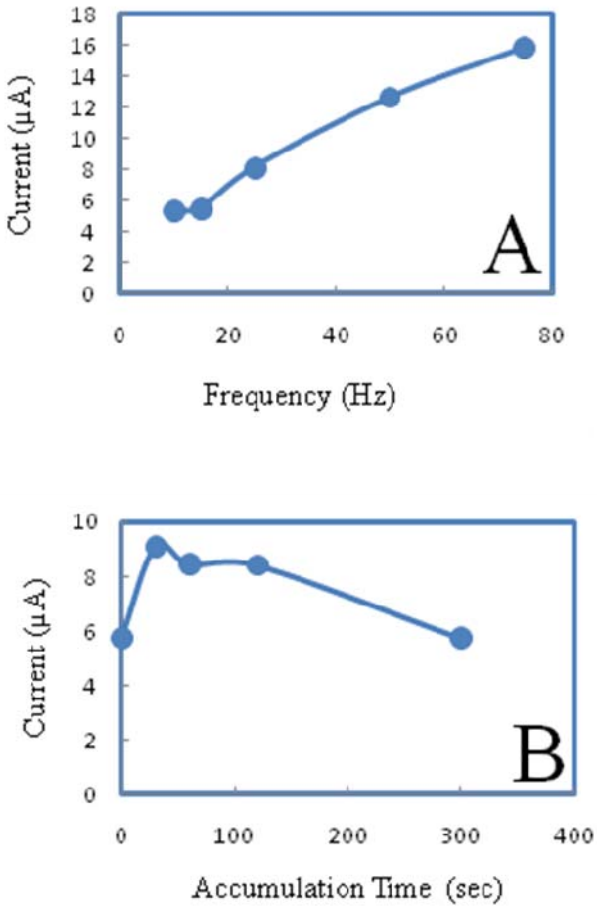


Figure 3.11. *Effect of frequency (A) and accumulation time (B) upon the square-wave stripping voltammetric response of 5.0 ppm guanine using GCE. Measurements were performed in phosphate buffer solution (0.2 M, pH6.0). Other conditions, as in Figure 3.10.*

3.2.2.3.3. Analytical Determination

In order to assess the performance of our system for guanine sensing, we have established a calibration curve. Figure 3.12 plays the calibration curve of guanine at glassy carbon electrode surface where the signal increases linearly in the range of 0.5-15 ppm, then levels off. A detection limit is estimated as well, 100 ppb (0.66 µmol/l) according to the signal/noise ratio equal to three.

After a series of seven measurements of 1.0 ppm guanine the relative standard deviation is calculated to be 0.3689 as shown in Figure 3.13.

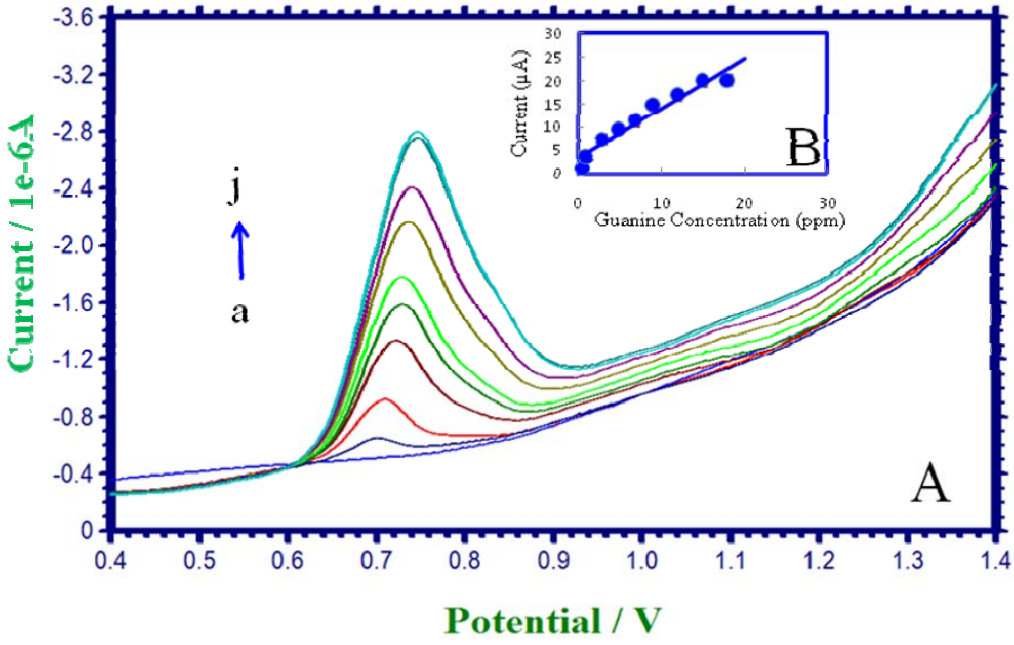


Figure 3.12. *(A) Square-wave stripping voltammograms of guanine concentrations using GCE: 0.0(a), 0.5(b), 1.0(c), 3.0(d), 5.0(e), 7.0(f), 9.0(g), 12(h), 15(i), and 18(j) ppm. Measurements were performed in phosphate buffer solution (0.2 M, pH6.0). Accumulation time, 30 sec at +0.5V. Potential step, 4 mV; Frequency, 25 Hz; Pulse amplitude, 50 mV. (B) The corresponding calibration plot.*

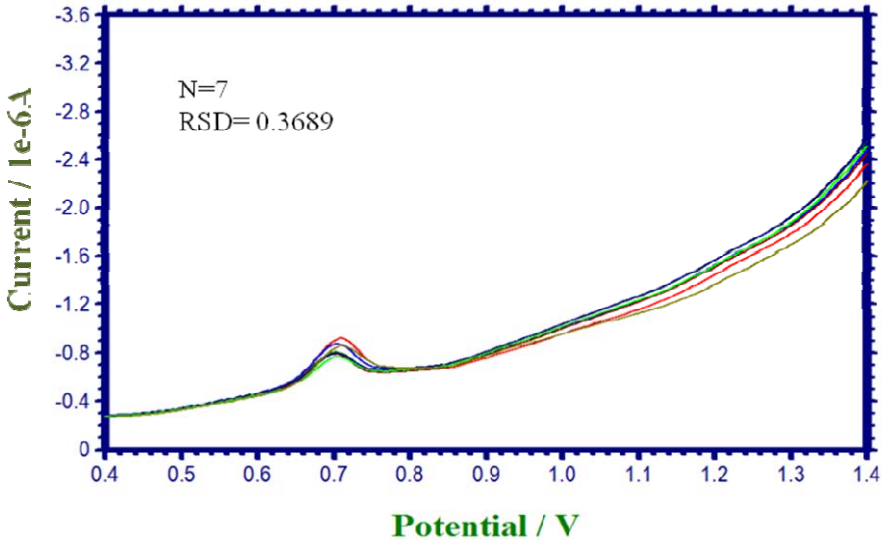


Figure 3.13. *Square-wave stripping voltammograms of 1.0 ppm guanine using GCE. a series of measurements of seven different guanine samples at a new surface every time. Other conditions, as in Figure 3.12.*

3.2.3. Using Graphite Pencil Electrode Surfaces

3.2.3.1. Electrode Preparation

A pencil Model P205 (Pentel, Japan) was used as a holder for the pencil lead. The electrical contact with the lead was achieved by soldering a metallic wire to the metallic part that holds the lead in place inside the pencil. The pencil was fixed vertically with 11 mm of the pencil extrude outside and 6.0 mm of the lead that immerses in the solution. Such length corresponds to an active electrode area of about 9.82 mm^2.

3.2.3.2. Procedure

The pencil was fixed vertically with 9.82 mm^2 electrode surface area immersed in the solution. A short (30s) electrochemical pre-treatment at +1.4 V (in a blank phosphate buffer solution; 0.2 M, pH 6.0) preceded the measuring step 60s accumulation at +0.5 V in a stirred solution of 0.2 M phosphate buffer (pH6.0) containing a specific concentration of guanine. The stirring was then stopped for 5s; this was followed by a subsequent stripping using a square wave voltammetric waveform with a 4 mV potential step, a 25 Hz frequency and an amplitude of 50 mV.

3.2.3.3. Results and Discussion

3.2.3.3.1. Analytical Determination

In this part we have evaluated the behavior of guanine at the graphite pencil electrode surfaces from its calibration curve. As shown in Figure 3.14, the response increases linearly in the range of 3.0- 20.0 ppm.

According to S/N ratio equal to three, a detection limit of 35 ppb (0.22 µmol/l) is calculated from a well defined peak for a concentration of guanine as low as 100 ppb, such detection limit corresponds to 35.0 ppb.

The calculated relative standard deviation is 0.4921 for seven measurements of 5.0 ppm guanine (not shown).

In Table 3.2, we compare the results related to detection limits (D.L) and relative standard deviations (RSD) for the guanine at the studied different carbon electrode surfaces, (GCPE, GCE, and GPE). For both parameters (D.L and RSD) glassy carbon paste electrode get the best results.

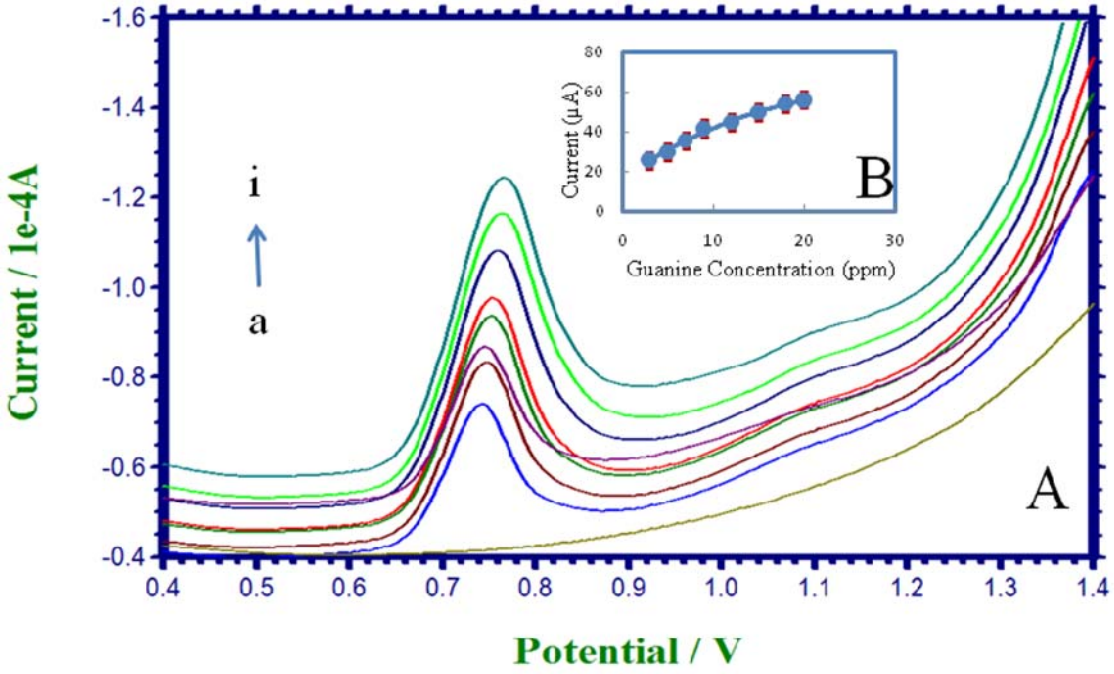


Figure 3.14. *(A) Square-wave stripping voltammograms of guanine concentrations using GPE: 0.0 (a), 3.0 (b), 5.0 (c), 7.0 (d), 9.0 (e), 12 (f), 15 (g), 18 (h), and 20 (i) ppm. Measurements were performed in phosphate buffer solution (0.2 M, pH6.0). Electrode pretreatment, 30 sec at +1.4 V; Accumulation time, 60 sec at +0.5V. Potential step, 4 mV; Frequency, 25 Hz; Pulse amplitude, 50 mV. (B) The corresponding calibration plot.*

Table 3.2. The detection limits and relative standard deviations for guanine at glassy carbon paste (GCP), glassy carbon (GC), and graphite pencil (GP) electrodes.

Electrode	**Detection Limit (µmol/l)**	**Relative Standard Diviation**
GCPE	0.11	0.1680
GCE	0.66	0.3689
GPE	0.22	0.4921

3.3. Electrochemical Investigation and Analytical Determination of Guanosine

3.3.1.Using Glassy Carbon Paste Electrode Surfaces

3.3.1.1. Reagents

Guanosine, sodium acetate and phosphate buffer solutions with different pHs, were obtained from Sigma. Glassy carbon spherical powders (20 – 50 μm) were obtained from Alfa Aesar (Ward Hill, MA, USA). The mineral oil was obtained from Aldrich. Copper atomic absorption standard solutions (containing 1000 μg/ml Cu(II), with 0.5 mol/l HNO_3), cadmium atomic absorption standard solutions (containing 1000 μg/ml Cd(II), with 0.5 mol/l HNO_3), lead atomic absorption standard solutions (containing 1000 μg/ml Pb(II), with 0.5 mol/l HNO_3), were purchase from Aldrich. Mannitol and EDTA was obtained from BDH Analar (Poole, England), lactose was purchased from B.B.L (Baltimore, Maryland, USA), sodium dodecylbenzene sulfonate was purchased from Science lab. Com (Houston, Texas, USA), while ascorbic acid was obtained from Fluka AG (Buchs, Switzerland). All stock solutions were prepared using deionized water.

3.3.1.2. Procedure

Cyclic voltammetry (CV) measurements were performed by treating the surface of the electrode at +1.7 V for 60s followed by 120s accumulation at +0.4 V in a stirred solution of 0.2 M phosphate buffer (pH6.0) (or as mentioned otherwise) containing a specific concentration of guanosine. Then the potential was scanned in the range (-0.2 - 1.6) V in a quisent solution; quiet time 5s.

Using square wave voltammetry (SWV), measurements were performed by treating the surface of the electrode at +1.7 V for 60s followed by 120s accumulation at

+0.4 V in a stirred solution of 0.2 M phosphate buffer (pH6.0) containing a specific concentration of guanosine. The stirring was stopped for 5s quiet time; which was followed by a subsequent stripping using a square wave voltammetric waveform, with a 4.0 mV potential step, 100 Hz frequency and amplitude of 50 mV (or as mentioned otherwise). A new glassy carbon paste surface was used in every measurement. The electrode surface was smoothed and rinsed carefully with deionized water prior to each measurement.

3.3.1.3. Results and Discussion

The present study evaluates the use of a new material/transducer, glassy carbon paste composite material, for guanosine analytical determination.

3.3.1.3.1. Electrochemical Investigation

The fourth analyte we are investigating in this work is guanosine, the nucleoside of the guanine base. Generally, the nucleosides are expected to be oxidized at more positive potentials than their bases.

Figure 3.15 shows the cyclic voltammogram for 1.0 ppm guanosine, where one oxidation peak with an irreversible process appeared at hiegher potential (+1.05V) than guanine (+0.81V).

To explore the adsorptivity of the guanosine nucleoside at the GCPE surfaces, we plot the obtained CV current vs. (U). A straight line was obtained, Figure 3.16B, confirming the adsorption phenomena guanosine moiety.

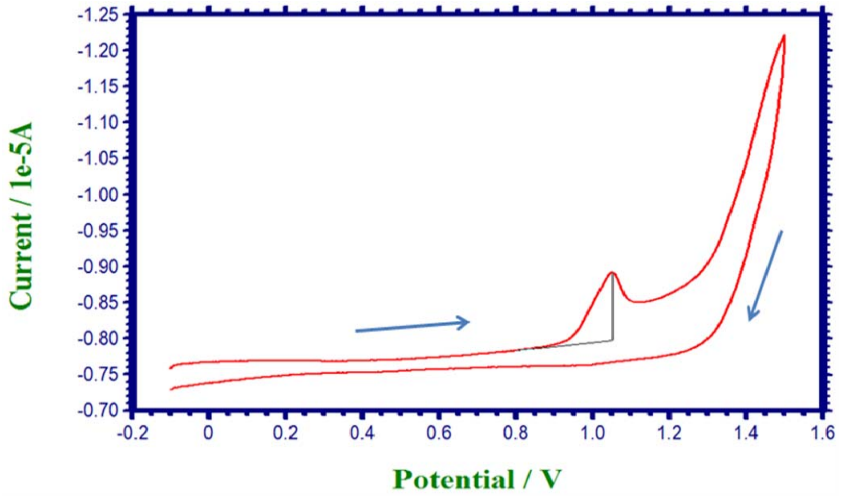


Figure 3.15. *Cyclic voltammogram of 1.0 ppm guanosine using GCPE at scan rate 100 mV/s. Measurements were performed in phosphate buffer solution (0.2 M, pH6). Electrode pretreatment 1.0 min at +1.7 V. Accumulation time 2.0 min at +0.4V.*

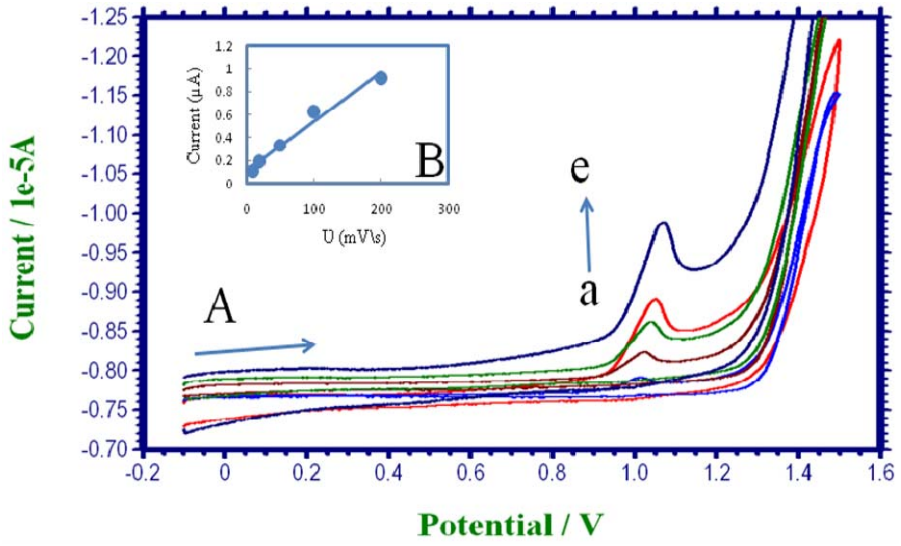


Figure 3.16. *(A) Cyclic voltammograms of 1.0 ppm guanosine using GCPE with different scan rates, 25 (a), 50 (b), 75 (c), 100 (d), and 200 (f) mV/s. (B) The corresponding plots. Other conditions, as in Figure 3.15.*

3.3.1.3.2. Optimization

Figure 3.17 shows the square-wave stripping voltammetric responses of 1.0 ppm guanosine at the fabricated glassy carbon paste electrode (GCPE) in two different buffers (acetate and phosphate buffers) and at different pHs. The guanosine electrochemical response in phosphate medium was more predominate from the response in acetate as shown in Figure 3.17 (a, b) vs. Figure 3.17 (c, d and e). There is a cathodic potential shift for the guanosine oxidation peak position with the increase of the pH. Within the same phosphate medium, that means that protons were involved in the electrode reaction. By plotting the Epa vs. pH, a good linear relationship was established with the linear regression equation as:

$$E_{pa}\,(V) = -0.046pH + 1.24 \qquad (R^2 = 0.981) \ldots\ldots\ldots\ldots\ldots\ldots \text{(Eq. 3.2)}$$

pH 6.0 was the optimum pH among all examined pHs with respect to the height and shape of the obtained guanosine oxidation peak.

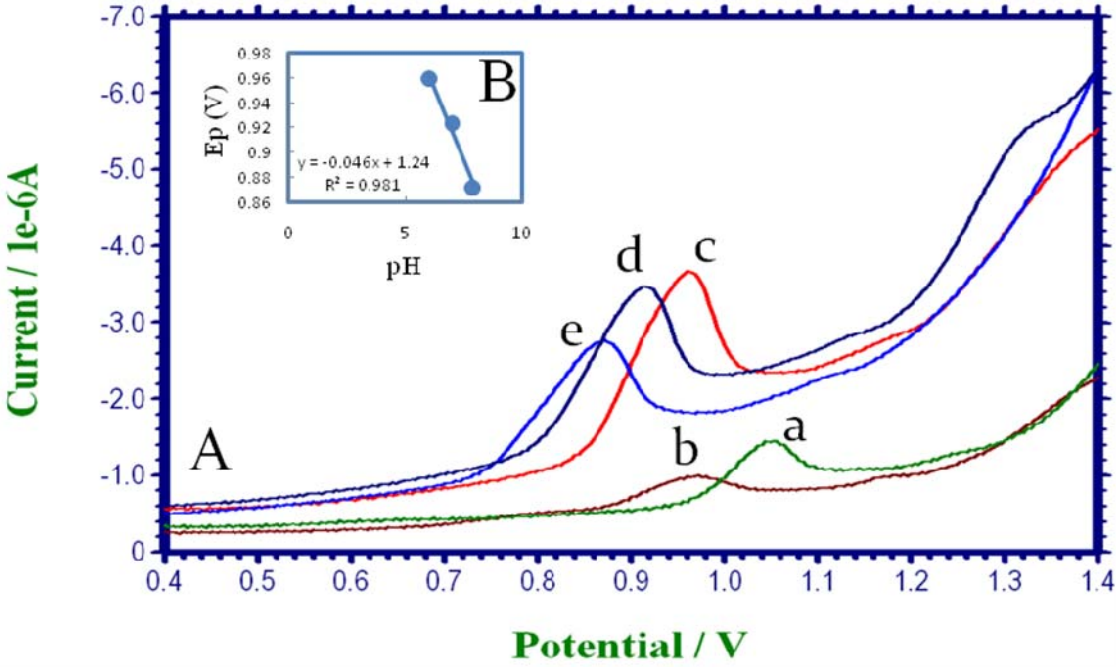


Figure 3.17. *Square-wave stripping voltammograms of 1.0 ppm guanosine using GCPE in different buffer media with different pHs: 0.2M acetate buffer, pH 4.0 (a), pH 6.0 (b); 0.2 M phosphate buffer, pH 6.0 (c), pH 7.0 (d), pH 7.9 (e). Electrode pretreatment, 1.0 min at +1.7 V; Accumulation time, 2.0 min at +0.4V. Potential step, 4 mV; Frequency, 25 Hz; Pulse amplitude, 50 mV.*

The dependence of the square wave voltammetric oxidation peak of guanosine was studied at different pulse amplitude as shown in Figure 3.18A, and from the corresponding plot, (Figure 3.18B) the peak increases linearly up to 50 mV, and levels off thereafter. For that 50 mV was chosen for the optimum conditions. It was found also that the larger the frequency, the higher the guanosine peak. Figure 3.19 shows the voltammograms and the corresponding plot of the recorded for guanosine oxidation current at different frequencies. The frequency value, 100 Hz, was chosen where a highly symmetric appearance and smooth peak obtained. Frequencies above 100 mV cause noise for the guanosine oxidation peak. A large noise level was observed without the electrochemical activation. New paragraph Figure 3.20A, shows the effect of the accumulation potential. No much differences in the recorded peak when the accumulation potential changes from -0.2 to +0.4V (*vs.* Ag/AgCl Sat.KCl), and the signal decreases at higher anodic potential. For that +0.4V was chosen as an optimum accumulation potential. Accumulation time was tested as well on the guanosine response. Figure 3.20B, shows the influence of the accumulation time where the recorded signal increases rapidly with the accumulation time up to 120 sec, then started to decrease slowly thereafter. Such profile could be due to the possible surface saturation of glassy carbon paste with guanosine molecules. Two minutes accumulation time was chosen. For subsequent experiments.

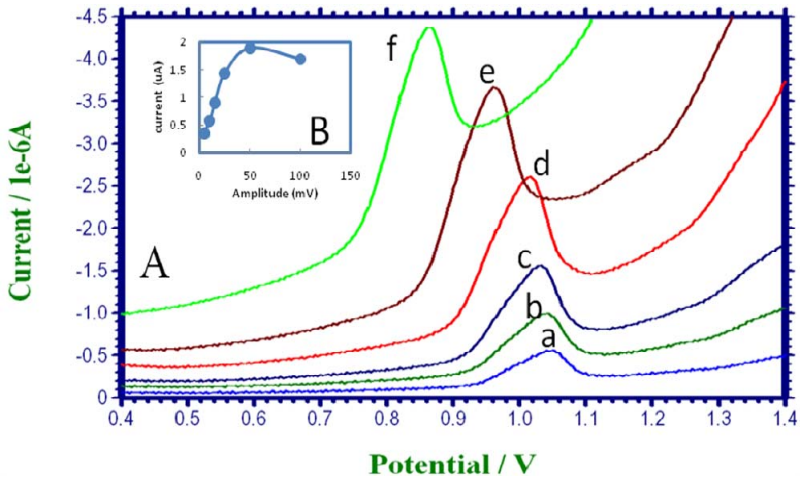


Figure 3.18. *(A) Square-wave stripping voltammograms of 1.0 ppm guanosine using GCPE at different amplitudes: 5 (a), 10 (b), 15 (c), 25 (d), 50 (e), and 100 (f) mV. (B) The corresponding plot . Other conditions, as in Figure 3.17.*

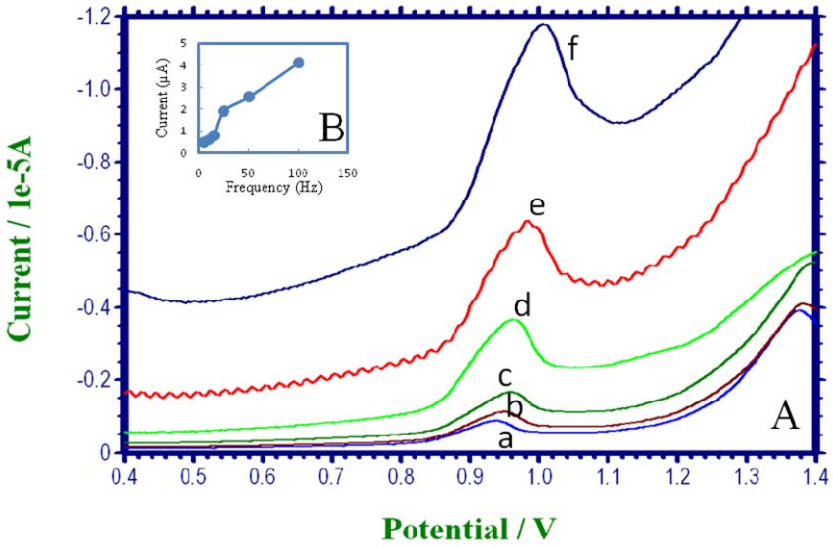


Figure 3.19. *(A) Square-wave stripping voltammograms of 1.0 ppm guanosine using GCPE at different frequencies: 5 (a), 10 (b), 15 (c), 25 (d), 50 (e), and 100 (f) Hz. (B) The corresponding plot. Other conditions, as in Figure 3.17.*

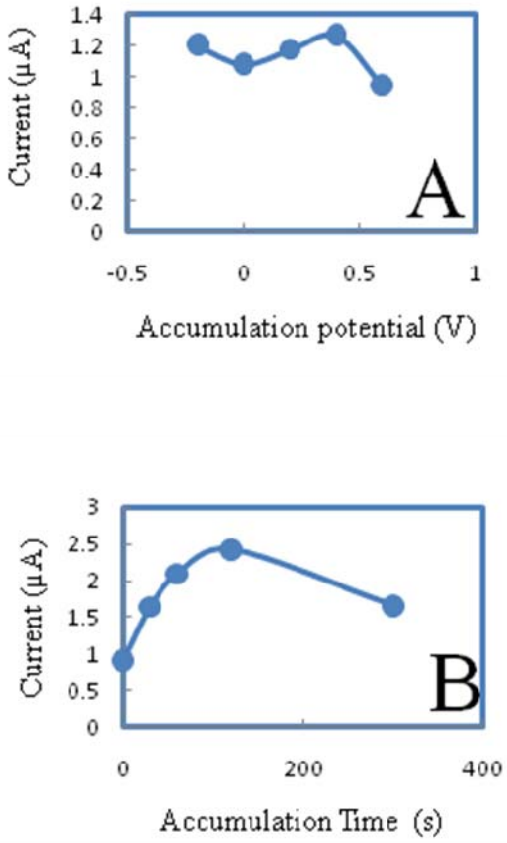


Figure 3.20. *Effect of accumulation potential (A), and accumulation time (B) upon the Square-wave stripping voltammetric response of 1.0 ppm guanosine using GCPE. Measurements were performed in phosphate buffer solution (0.2 M, pH6.0). Other conditions, as in Figure 3.17.*

3.3.1.3.3. Analytical Determination

The optimum conditions for electroanalytical determination of guanosine at the new composite material, glassy carbon paste (GCP), by square wave voltammetry were found as on the following: buffer solution, 0.2 M phosphate; pH, 6.0; adsorption accumulation potential, +0.4 V; adsorption accumulation time, 120 sec; pulse amplitude, 50 mV; frequency, 100 Hz. Figure 3.21 shows the calibration study under these optimum conditions. With the increase of the guanosine concentration, the peak current increases linearly within the concentration range 0.25 to 5.0 ppm, (Figure 3.21A). Further increase in the guanosine concentration deviates from linearity as shown in the corresponding calibration plot, Figure 3.21B. This is due to a complete coverage of the electrode surface as the concentration increases. Measurements of a 100 ppb guanosine solution were used to estimate the detection limit. A value of around 35 ppb can thus be estimated based on the signal-to-noise characteristics of these data (S/N = 3). Such detection limit corresponds to 0.12 μmol/l.

A series of seven measurements of 1.0 ppm guanosine yielded a highly reproducible response with a relative standard deviation (RSD) of 0.2899.

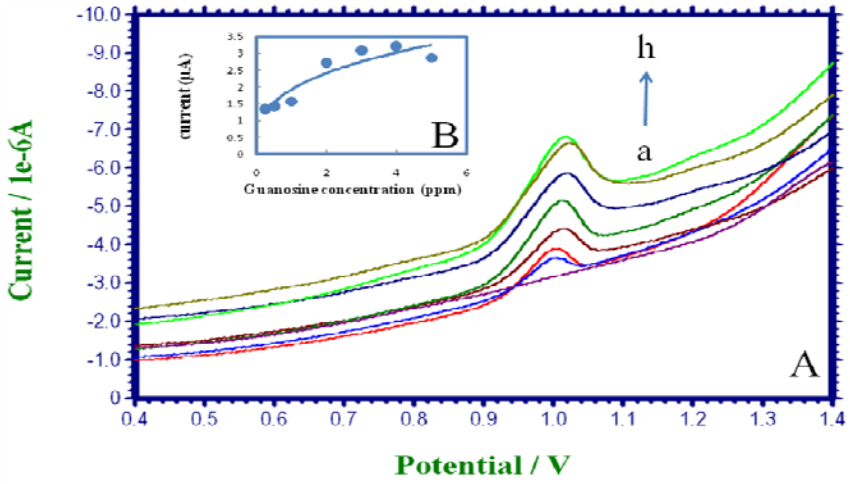


Figure 3.21. *(A) Square-wave stripping voltammograms of guanosine concentrations using GCPE : 0.0 (a), 0.25 (b), 0.5 (c), 1.0 (d), 2.0 (e), 3.0 (f), 4.0 (g) and 5.0 (h) ppm. Measurements were performed in phosphate buffer solution (0.2 M, pH6.0). Electrode pretreatment, 1.0 min at +1.7 V; Accumulation time, 2.0 min at +0.4 V. Potential step, 4 mV; Frequency, 100 Hz; Pulse amplitude, 50 mV. (B) The corresponding calibration plot.*

3.3.1.3.4. Interferences

To know the effect of heavy metals on the guanosine response we have tested the following metals (Pb, Cd, and Cu) by adding to guanosine sample, as a representive of all metals, 2.0 ppm copper ions, Cu(II) was added to 5.0 ppm guanosine. An increase in the guanosine peak was observed yet much less than that observed for guanine. Figure 3.22 shows the related square wave voltammograms. Where Figure 3.22a, shows the guanosine response in absence of Cu(II), Figure 3.22b, shows the response in presence of 2.0 ppm Cu(II).

The evaluation of the selectivity of the proposed method was performed by examining different organic compounds with different concentrations under the same experimental conditions. Table 3.3 shows the influence of adding different concentrations of the additive compounds. There is no interference at concentration 1.0 ppm of ascorbic acid and less than 4% when adding 5.0 ppm . While small decreasing appeared by adding same concentration of other compounds. The guanosine response decreases gradually between 1.0 and 5.0 ppm of other compounds.

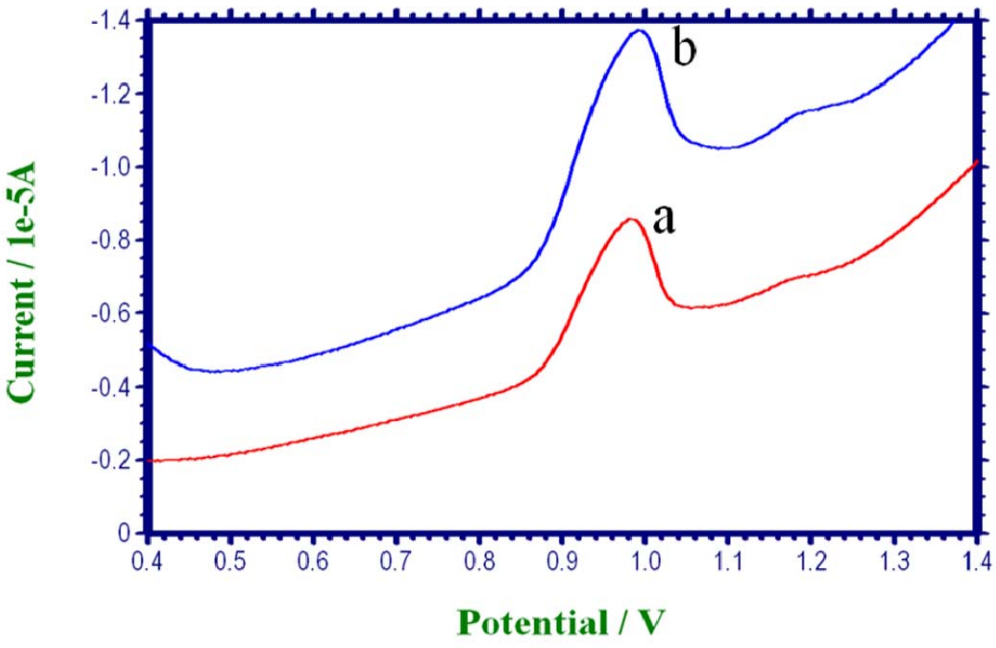


Figure 3.22. *Square-wave stripping voltammograms of 5.0 ppm guanosine in absence (a) and in presence (b) of 2.0 ppm Cu(II) using GCPE. Measurements were performed in phosphate buffer solution (0.2 M, pH6.0). Electrode pretreatment, 1.0 min at +1.7 V; Accumulation time, 2.0 min at -0.05V. Potential step, 4 mV; Frequency, 100 Hz; Pulse amplitude, 50 mV.*

Table 3.3. Influence of interferences on the determination of 5.0 ppm guanosine.

Interference	Concentration/ppm	Change of Signal (%)
Ascorbic Acid	1	0.0
	3	1.2
	5	3.4
Lactose	1	18.7
	3	18.7
	5	20.5
SDBS	1	17.7
	3	39.9
	5	41.4
Mannitol	1	6.7
	3	18.7
	5	29.5
EDTA	1	10.9
	3	26.01
	5	48.2

3.3.1.3.5. Application

The developed detection method using the glassy carbon paste composite material was tested on the detection of guanosine in human urine. Representative square-wave stripping voltammogram for trace determination of guanosine (1.0 ppm) spiked in human urine was illustrated in Figure 3.23. A defined guanosine signal was obtained as shown in Figure 3.23, b, corresponding to the 1.0 ppm guanosine concentration.

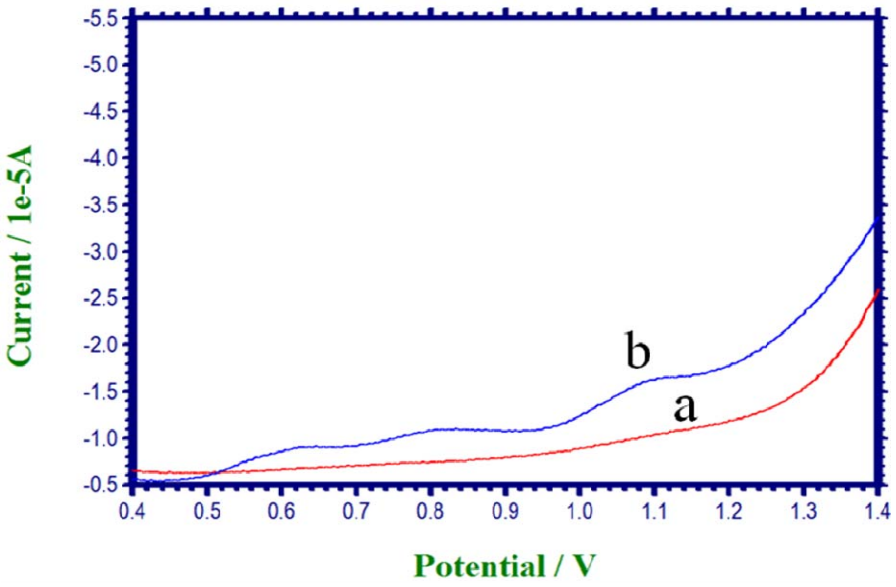


Figure 3.23. *Square-wave stripping voltammogram of 1.0 ppm guanosine using GCPE in Urine medium. Other conditions, as in Figure 3.21.*

3.3.2. Using Glassy Carbon Electrode Surfaces

3.3.2.1. Procedure

Before using the glassy carbon electrode, it was polished with alumina powder and rinsing with deionized water prior to each measurement. This was followed by a 30s accumulation at +0.4 V in a stirred solution of 0.2 M phosphate buffer (pH6.0) containing a specific concentration of guanosine, the stirring was then stopped for 5s and followed by a subsequent stripping using a square wave voltammetric waveform, with a 4 mV potential step, 25 Hz frequency and amplitude of 50 mV.

3.3.2.2. Results and Discussion

3.3.2.2.1. Electrochemical Investigation

The type of carbon electrodes under investigation doesn't affect the electrochemical behavior of the analyte too much. In here we have used another carbon electrode, GCE, for the guanosine electrochemical investigation, as expected similar behavior was obtained using (GCPE), (Figure 3.24). One oxidation peak with an irreversible process took place at the surface of the electrode.

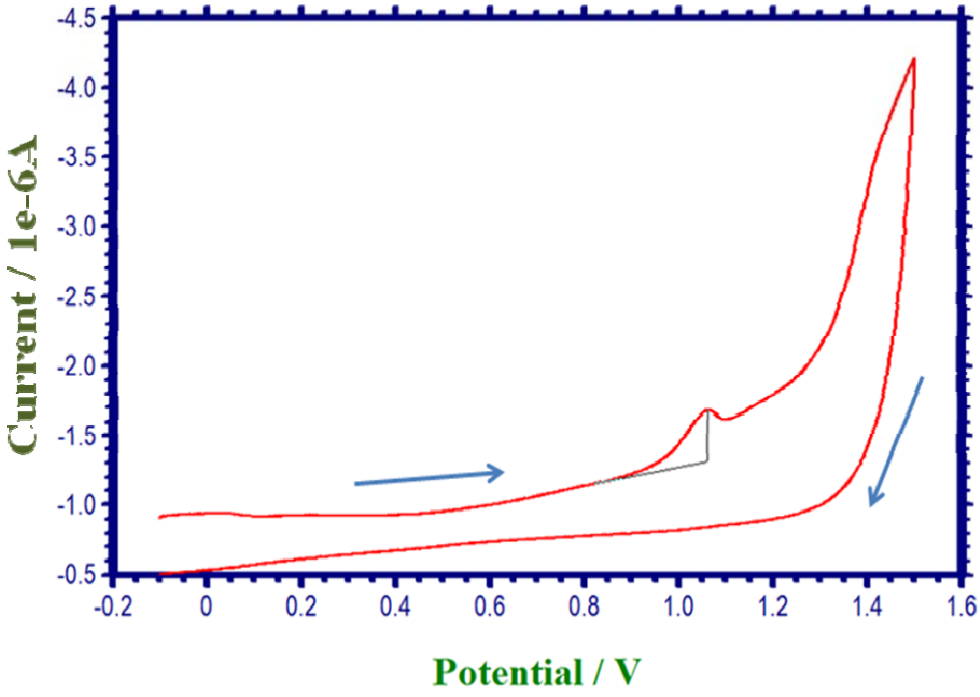


Figure 3.24. *Cyclic voltammograms of 5.0 ppm guanosine using GCE at scan rate 100 mV/s. Measurements were performed in phosphate buffer solution (0.2 M, pH6). Accumulation time 30 min at +0.4V.*

3.3.2.2.2. Optimization

The objective of this part is to optimize the parameters affecting the stripping voltammetric responses of guanosine at glassy carbon electrode surface.

We assessed the role of different pulse amplitudes, different frequencies and the effect of different accumulation time.

Figure 3.25A, reflects the pulse amplitude effect, the response increases nearly linearly up to 50 mV then start decreasing so it is chosen as optimum.

While Figure 3.25B, shows peak current plot after applying different frequencies, the peak increases with increasing the frequency, with consideration of the best shape, 25 Hz is chosen as a frequency for the subsequent work.

In Figure 3.25C, the effect of accumulation time was studied. No big difference in the height of guanosine signal was observed in the range 30-120 sec, then the signal started decreasing at longer accumulation time. So 30 sec accumulation time was employed as optimum.

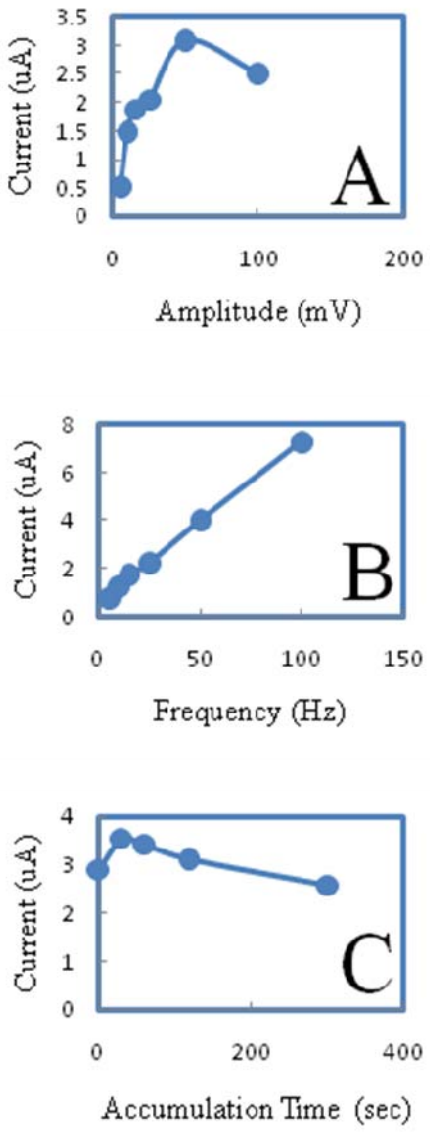


Figure 3.25. *Effect of pulse amplitude (A), frequency (B), and accumulation time (c) upon the Square-wave stripping voltammetric response for 5.0 ppm guanosine using GCE. Other conditions, as in Figure 3.24.*

3.3.2.2.3. Analytical Determination

The linearity could be established for guanosine at GCE surface in the range of 1.0 to 8.0 ppm. Figure 3.26A, shows the square wave voltammograms for different concentrations of guansine. As shown on corresponding plot (Figure 3.26B), the peak current increases slowly with good linearty up to 8.0 ppm then levels off. The relative standard deviation was calculated for seven measurments to be 0.2841. the detection limit is 84.9 ppb (0.3 µmol/l) estimated depending on the (S/N=3).

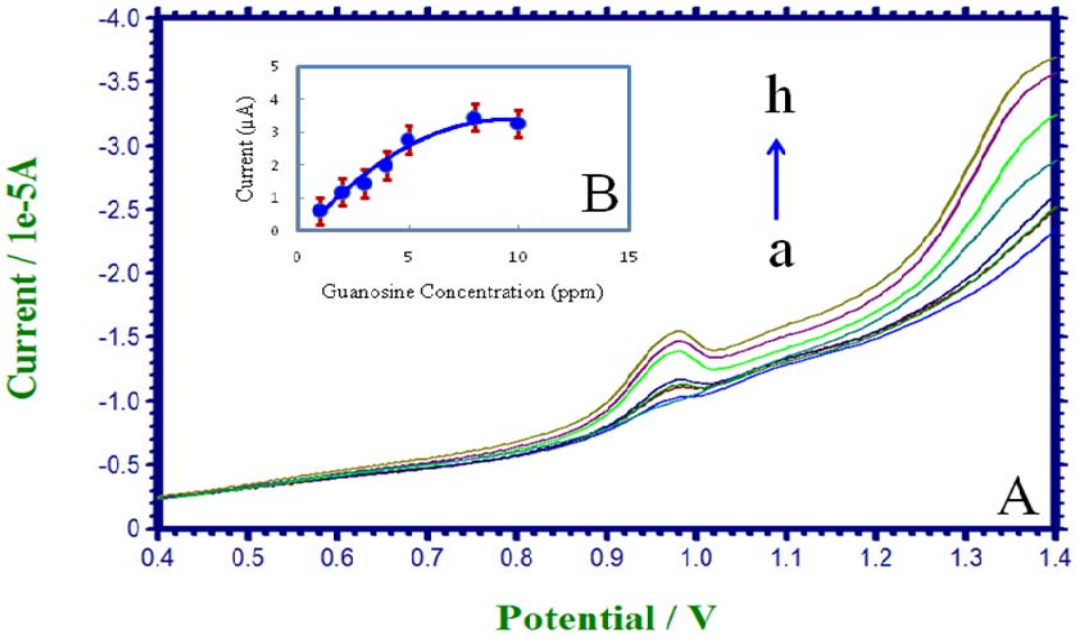


***Figure* 3.26.** *(A) Square-wave stripping voltammograms of guanosine concentrations using GCE: 0.0 (a), 1.0 (b), 2.0 (c), 3.0 (d), 4.0 (e), 5.0 (f), 8.0 (g), and 10 (h) ppm. Measurements were performed in phosphate buffer solution (0.2 M, pH6.0). Accumulation time, 30 sec at +0.4V. Potential step, 4 mV; Frequency, 25 Hz; Pulse amplitude, 50 mV. (B) The corresponding calibration plot.*

3.3.3. Using Graphite Pencil Electrode Surfaces

3.3.3.1. Procedure

The pencil was fixed vertically with 11 mm of the pencil extrude outside and 6.0 mm of the lead that was immersed in the solution. A short (30 s) electrochemical pretreatment at +1.4 V (using the blank phosphate buffer solution; 0.2 M, pH 6.0) preceded the measuring step of 60s accumulation at +0.4 V in a stirred solution of 0.2 M phosphate buffer (pH6.0) containing a specific concentration of guanosine. The stirring was then stopped for 5s which was followed by a subsequent stripping using a square wave voltammetric waveform, with a 4.0 mV potential step, 25 Hz frequency and pulse amplitude of 50 mV.

3.3.3.2. Results and Discussion

3.3.3.2.1. Analytical Determination

Figure 3.27 shows the calibration study under the optimum conditions. With the increase of the guanosine concentration, the peak current increases linearly within the concentration range 1.0 to 10.0 ppm. Further increase in the guanosine concentration deviates from linearity appeared as shown in the Figure 3.27B. This is due to a complete coverage of the electrode surface as the concentration increases.

Measurements of a 150 ppb guanosine solution were used to estimate the detection limit. A value of around 50 ppb can thus be estimated based on the signal-to-noise characteristics of these data (S/N = 3). Such detection limit corresponds to 0.17 μmol/l. A series of seven measurements of 5.0 ppm guanosine yielded a highly reproducible response with a relative standard deviation of 0.3462.

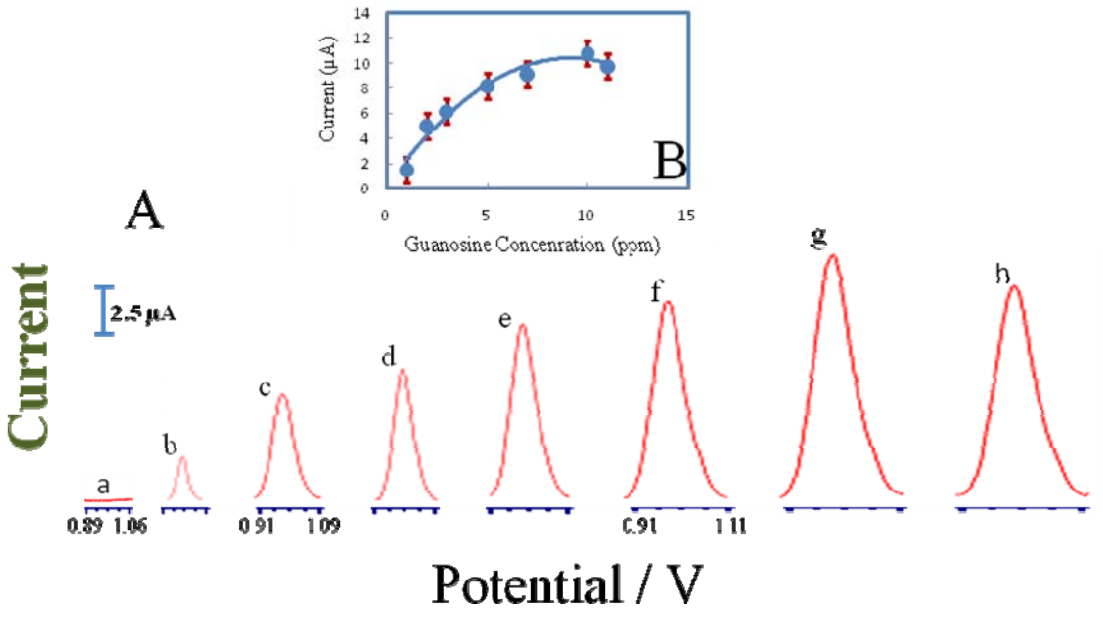


Figure 3.27. *(A) Square-wave stripping signals of guanosine at GPE: 0.0 (a), 1.0 (b), 2.0 (c), 3.0 (d), 5.0 (e), 7.0 (f), 10 (g), and 11(h) ppm. Measurements were performed in phosphate buffer solution (0.2 M, pH 6.0). Electrode pretreatment, 30 sec at +1.4 V; Accumulation time, 60 sec at +0.4V. Potential step, 4 mV; Frequency, 25 Hz; Pulse amplitude, 50 mV. (B) The corresponding calibration plot.*

All detection limits and relative standard deviations related to the guanosine oxidation at different carbon electrode surfaces (GCPE, GCE, GPE), are summarized in Table 3.4.

We could get the lowest detection limit at the glassy carbon paste electrode, with almost same relative standard deviation as glassy carbon electrode. So we can conclude as a result of that, GCPE is the best for the guanosine electroanalysis.

Table 3.4. The detection limits and relative standard deviations for guanosine at glassy carbon paste (GCPE), glassy carbon (GCE), and graphite pencil (GPE) electrodes.

Electrode	**Detection Limit (µmol/l)**	**Relative Standard Diviation**
GCPE	0.12	0.2899
GCE	0.30	0.2841
GPE	0.17	0.3462

CHAPTER 4

4. Dual and Simultaneous Determination of Nucleic Acid Constituents

4.1. Introduction

The separation and detection of nucleic acid constituents are interesting and challenging at the same time. This is due to the fact that these compounds are involved in a large number of biochemical processes.

To date, a series of chromatographic methods, including thin layer chromatography (TLC) (80, 81), gas chromatography (GC) (82, 83), high performance liquid chromatography (HPLC) (84- 91), ultra-performance liquid chromatography (UPLC) (92), ion-pair reverse-phase chromatography (IP-RPC) (93- 95), liquid chromatography–mass spectrometry (LC–MS) (96- 98), micellar electrokinetic chromatography (MEKC) (99, 100), and capillary electrochromatography (CEC) (101), have been developed for the individual, dual and simultaneous determination of nucleic acid constituents in biological fluids and herbal materials. Although the chromatographic methods are sensitive, yet they require sample pre-treatment and many time-consuming extraction steps. However, electrochemical determination has shown to be a powerful method for the detection of a wide range of analytes including several nucleic acid constituents. Electrochemical methods have many advantages: high sensitivity, good selectivity, rapid response, low cost and simplicity.

The aim of this section is mainly to use the developed electrochemical method for dual and simultaneous determination of nucleic acid constituents. To validate the

collected data, high performance liquid chromatography (HPLC) is used as another analytical determination method. HPLC is proven as a powerful technique for analysis of such compounds.

4.2. Apparatus

4.2.1. Electrochemical Methods

For electrochemical determination, voltammetric measurements were performed with an electrochemical work station (CHI660C, CH Instruments Inc, Austin, TX, USA). The Ag/AgCl reference electrode (in 3M KCl, CHI111, CH Instruments Inc), platinum wire counter electrode (CHI115, CH Instruments Inc) and glassy carbon paste electrode as a working electrode were inserted into the 1 ml glass cell through holes in its Teflon cover.

4.2.2. High Performance Liquid Chromatography (HPLC) Method

Using HPLC, all analyses were performed on a Waters 2695, Separations Module (Waters, MA, USA), a μBondapack C18 column (10μm 125 Å. 4.6 x 150 mm) was used, photodiode array (PDA), Water 2996, served as a detector.

4.3. Reagents

4.3.1. Electrochemical Methods

Adenine, guanine, adenosine, guanosine, ssDNA, acetate buffer and phosphate buffer were purchased from Sigma (St. Louis, USA). Glassy carbon spherical powders (20 – 50 μm) were obtained from Alfa Aesar (Ward Hill, MA, USA). The mineral oil and copper atomic absorption standard solutions (containing 1000 μg/ml Cu(II), with 0.5

mol/l HNO_3) were purchased from Aldrich. All solutions were prepared using deionized water.

4.3.2. High Performance Liquid Chromatography (HPLC) Method

Adenine, guanine, adenosine, guanosine, acetate buffer and methanol (HPLC Grade) were purchased from Sigma (St. Louis, USA). All solutions were prepared using deionized water.

4.4. Procedure

4.4.1. Electrochemical Methods

Voltammetry measurements were performed by treating the surface of the glassy carbon paste electrode at +1.7 V for 60s followed by 120s accumulation at +0.5 V in a stirred solution of phosphate buffer (0.2M, pH6.0) (or as mentioned otherwise) containing a specific concentration of each analyte. The stirring was then stopped for 5s; then followed by a subsequent stripping using a square wave voltammetric waveform, with a 4.0 mV potential step, 100 Hz frequency and amplitude of 50 mV. A new glassy carbon paste surface was used in every measurement. The electrode surface was smoothed and rinsed carefully with deionized water prior to every measurement.

4.4.2. High Performance Liquid Chromatography (HPLC) Method

For chromatograohic analysis, the samples were separated using an isocratic mobile phase (0.1M acetate buffer, pH=5, MeOH 4%). Flow rate was 1.5 ml/min, the injection volume was 45 µL. The peaks were detected at 260 nm. Reconditioning the column with the mobile phase for 10 min.

Stock solutions of each analyte were prepared by individually dissolving the available pure compound; adenine, guanine, adenosine, and guanosine, in deionized water. Calibration curves were generated from their solutions standards ranging from 50 ppb to 5.0 ppm.

4.5. Results and Discussion

Before doing the dual and simultaneous analysis for the compounds, a calibration curve for each analyte (guanine, adenine, guanosine, and adenosine) was done using HPLC. All analyses were performed on HPLC (Waters 2695, Separations Module), a μBondapack C18 column (10μm 125 Å. 4.6 x 150 mm) was used, photodiode array detector (PDA) was used as a detector, Water 2996, λ_{max}= 260 nm, mobile phase was a mixture of acetate buffer (0.1 M, pH 5.0), with MeOH 4% . Flow rate was 1.5 ml/min, volume injected was 45 μl.

Working stock solutions containing four reference compounds were prepared and diluted to appropriate concentrations for the construction of the calibration curves. Seven concentrations of the solution were analyzed in triplicate, and then the calibration curves were constructed by plotting the peak height versus the concentration of each analyte. The results were shown in Figure 4.1.

Peak identification was made on the basis of retention times. The standard addition method was also used to confirm peak identification.

Table 4.1 summarizes the linear correlation equations and correlation coefficients of guanine, adenine, guanosine and adenosine.

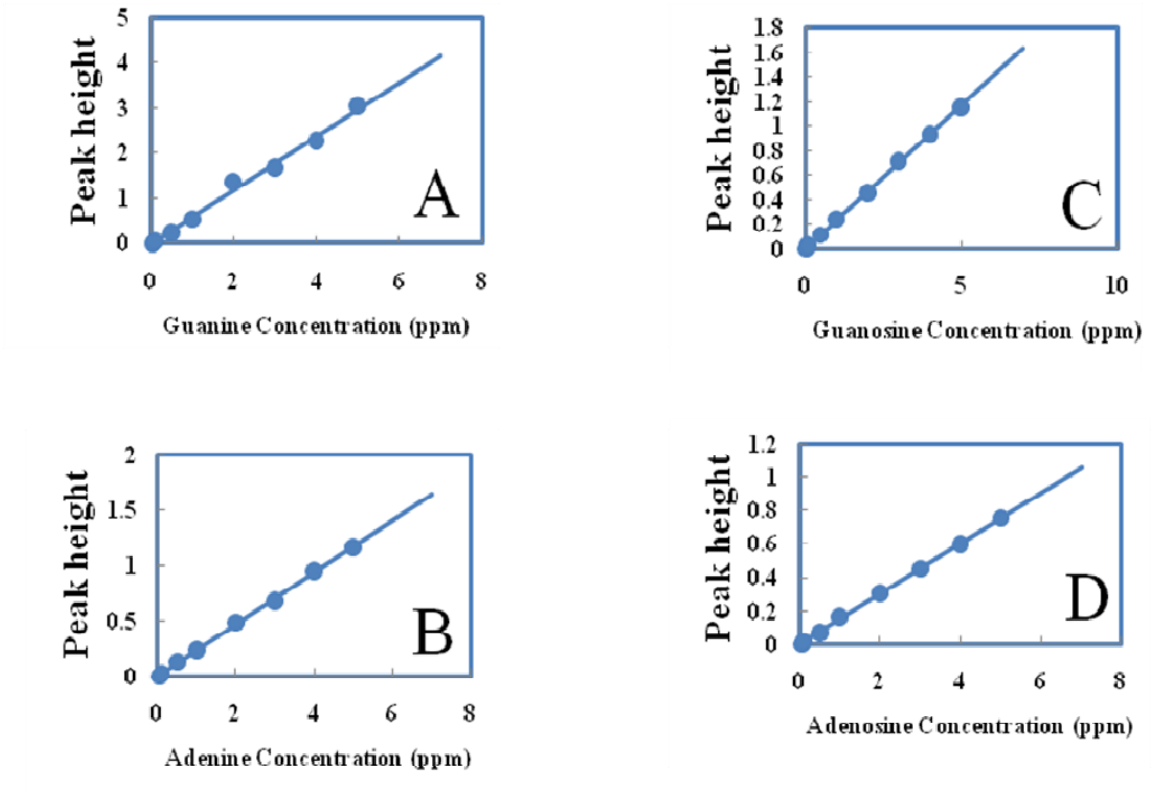


Figure 4.1. *Calibration curves of guanine (A), adenine (B), guanosine (C), and adenosine (D). Column, a µBondapack C18 column (10µm 125 Å. 4.6 x 150 mm), detector, photodiode array, (Water 2996, λ_{max}= 260 nm), mobile phase, a mixture of acetate buffer of 0.1 M, pH5.0, MeOH 4% .Flow rate, 1.5 ml/min, volume injected, 45 µl.*

Table 4.1. The linear correlations and correlation coefficients of guanine, adenine, guanosine and adenosine.

Compound	**Linear Correlation**	**Correlation Coefficient**
Guanine	Y = 0.5962x - 0.0167	0.9927
Adenine	Y = 0.2348x - 0.0005	0.9990
Guanosine	Y = 0.233x - 0.0001	0.9993
Adenosine	Y = 0.1508x + 0.0011	0.9995

* where Y is the relative peak height of the analyte, and x is the relative concentration of the analyte**.**

4.5.1. Dual Determination of Nucleic Acid Constituents

Multi-target assays, in which several targets are measured simultaneously in a single sample, represent several advantages and challenges as well. A considerable recent attention has been given to the development of different methods that permit simultaneous determination of two or more nucleic acid constituents in a single sample (102- 104). Little attention has been given to analogous electrochemical simultaneous detection (105- 107).

In this section we report on a dual determination protocols for pairs of nucleic acid constituents using different techniques.

4.5.1.1. Using Electrochemical Methods

Most efforts in this direction (dual determination) have been devoted to optical detection (102- 105). In here, we demonstrate new electrochemical methods for this purpose. Different electrochemical methods have been used: cyclic voltammetry (CV), square wave voltammetry (SWV), differential pulse voltammetry (DPV), and linear sweep voltammetry (LSV).

The performances of these electrochemical methods are illustrated in Figures 4.2 and 4.3 for a mixture of 1.0 ppm guanine and 1.0 ppm adenine at the glassy carbon paste electrode. Although well defined and separated peaks with all techniques have appeared, the obtained signals vary from one technique to another. With respect to the height of both peaks SWV is the best. For that SWV is used for the dual determination for all other possible pairs.

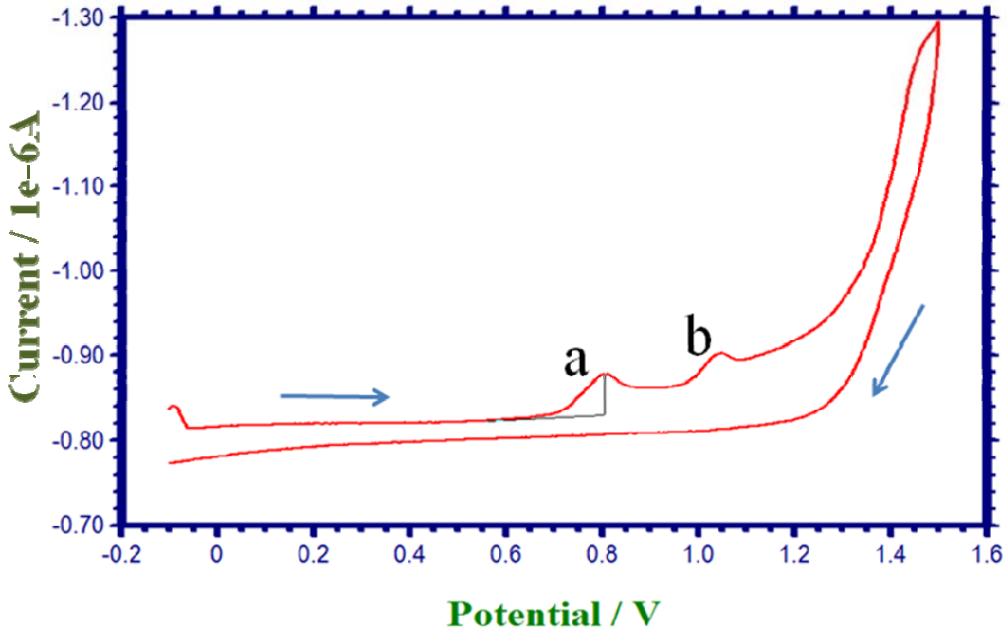


Figure 4.2. *Cyclic voltammogram of a mixture of guanine (a), and adenine (b) (1.0 ppm each) using GCPE. Measurements were performed in phosphate buffer solution (0.2 M, pH 6.0). Electrode pretreatment, 1.0 min at +1.7 V; Accumulation time, 2.0 min at +0.5V.*

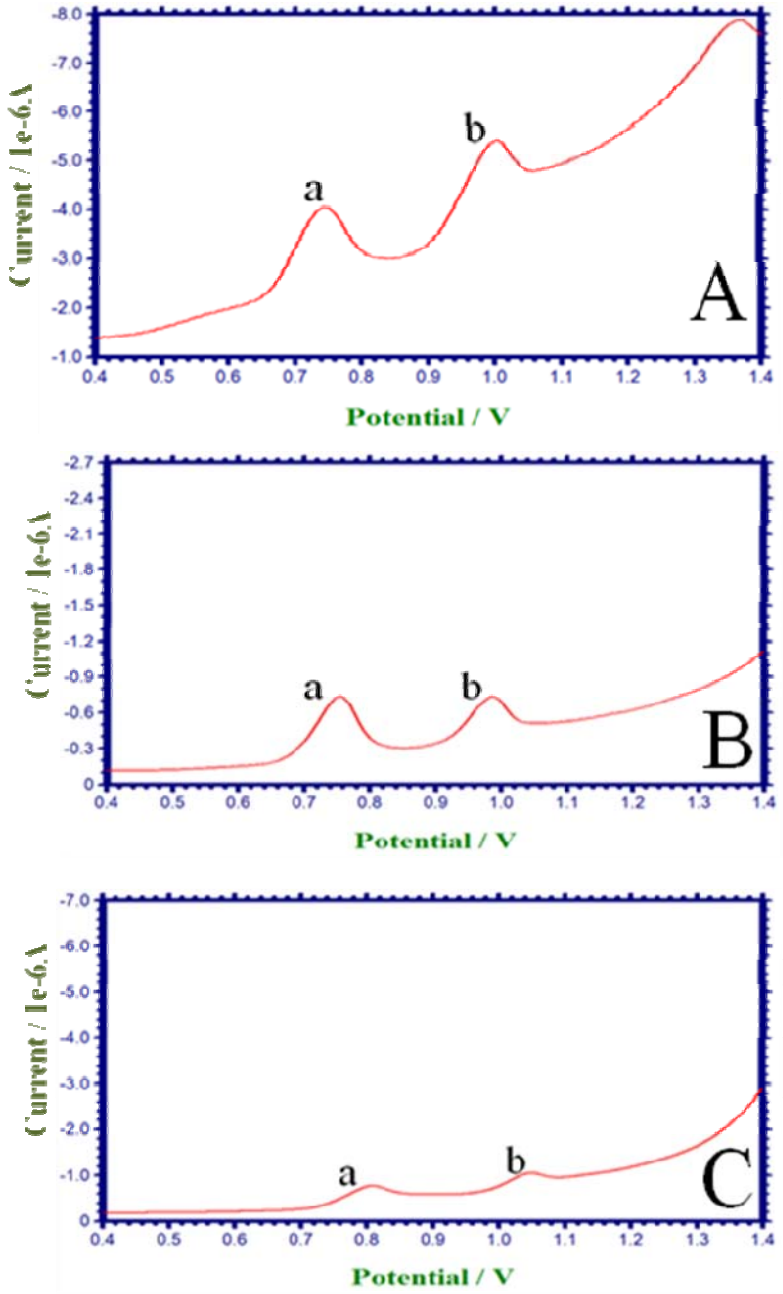


Figure 4.3. *Voltammograms of a mixture of guanine (a), and adenine (b) (1.0 ppm each) using SWV (A), DPV (B), LSV (C) at GCPE. Measurements were performed in phosphate buffer solution (0.2 M, pH 6.0). Electrode pretreatment, 1.0 min at +1.7 V; Accumulation time, 2.0 min at +0.5V. Potential step, 4 mV; Frequency, 100 Hz; Pulse amplitude, 50 mV.*

The anodic voltammograms for each possible pairs of nucleic acid constituents were recorded using square wave voltammetry at glassy carbon paste electrode. Successive well defined two peaks were observed. The voltammogram in Figure 4.4A, demonstrated a good resolution and peak height for a mixture of adenine and adenosine 5.0 ppm each. A mixture of guanine and guanosine is tested as well and shown in Figure 4.4B. In Figure 4.4C, the voltammogram of a mixture of guanosine and adenosine nucleosides in 5.0 ppm each is shown.

The developed detection method for the dual determination was tested as well on the detection of a mixture of guanine and adenine in human urine. Representative square-wave stripping voltammograms for trace determination of both analytes spiked in human urine were illustrated in Figure 4.5. Two defined peaks were obtained, corresponding to the 1.0 ppm guanine (a) and 1.0 ppm adenine (b) concentrations.

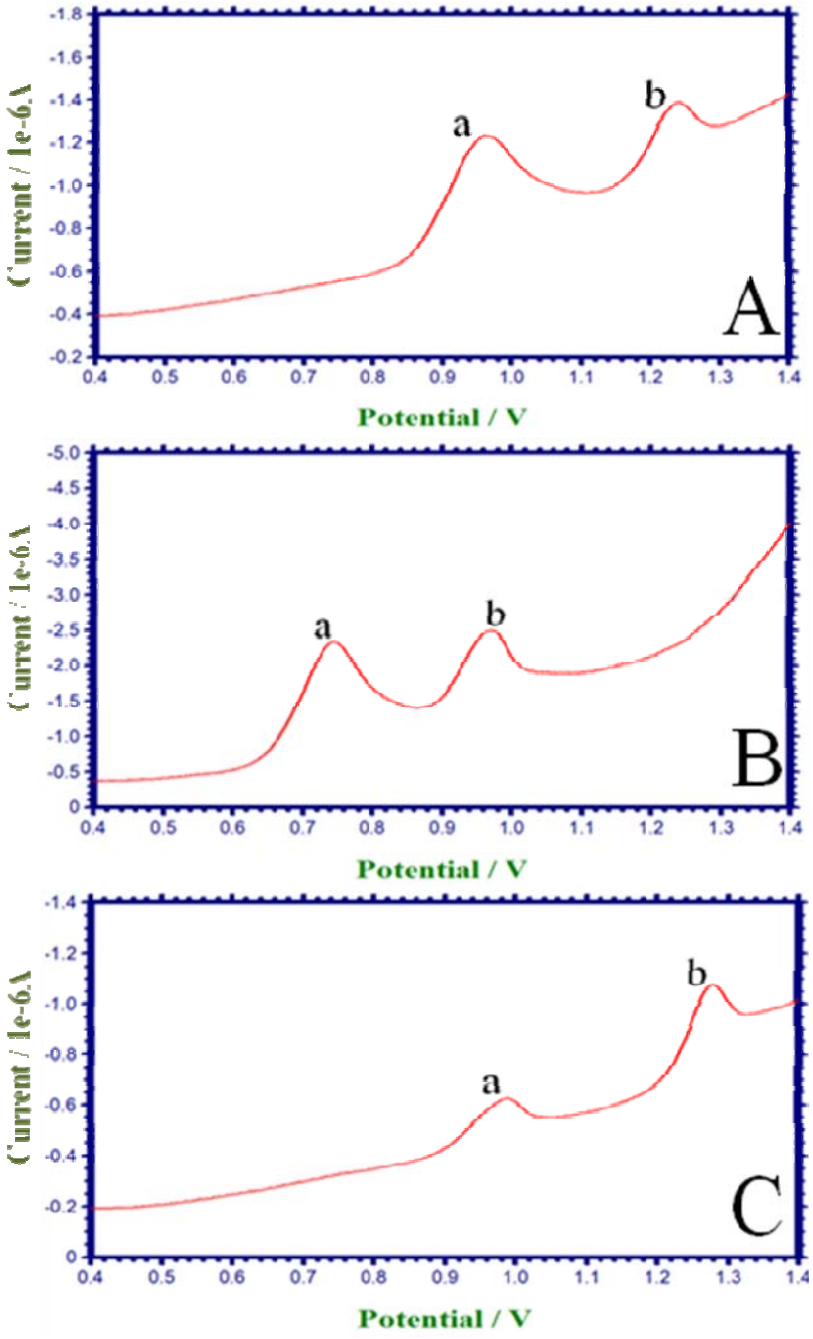


Figure 4.4. *Square-wave stripping voltammogram of a mixture of (A) adenine (a) and adenosine (b), (B) guanine (a) and guanosine (b), (C) guanosine (a) and adenosine (b), (5.0 ppm each) using GCPE. Other conditions, as in Figure 4.3.*

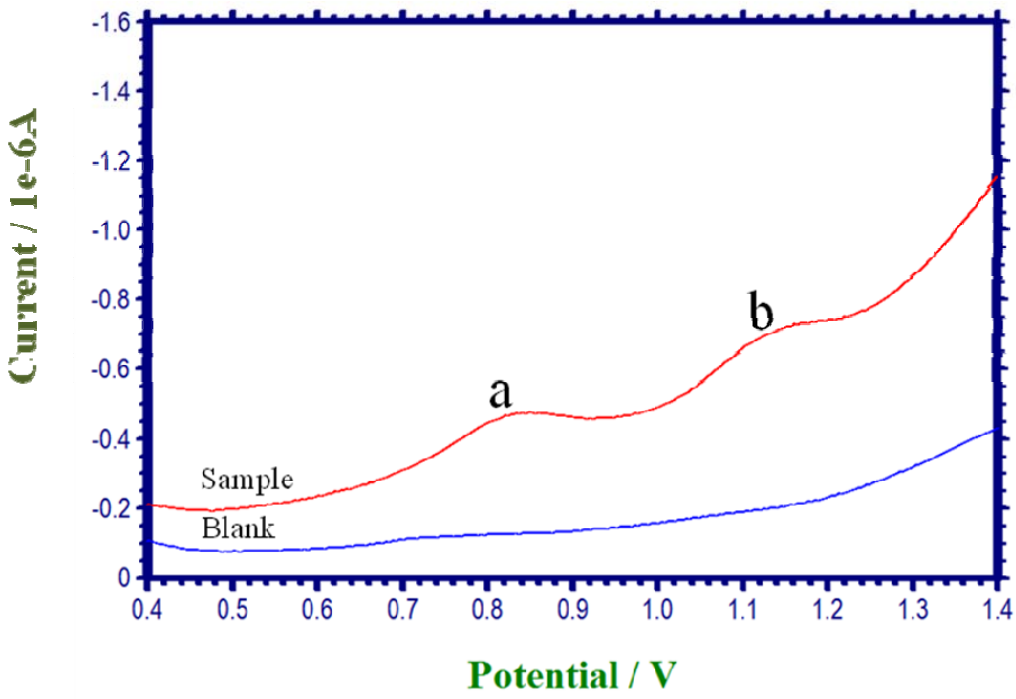


Figure 4.5. *Square-wave stripping voltammograms of a mixture of guanine (a) and adenine (b) (1.0 ppm each) using GCPE in Urine medium. Other conditions, as in Figure 4.3.*

4.5.1.2. Using High Performance Liquid Chromatography (HPLC) Method

The used chromatographic conditions allowed for the successful separation of pairs of nucleic acid constituents.

Figure 4.6 represents the chromatographic profiles for mixtures of (adenine + adenosine, A), (guanine + guanosine, B), (adenine + guanine, C) and (guanosine + adenosine, D) respectively.

Figure 4.6A, shows typical chromatogram of the analysis of 1.0 ppm adenine mixed with 1.0 ppm adenosine. While Figure 4.6B, displays the chromatogram of guanine and its nucleoside, guanosine, with concentration of 1.0 ppm for each. The two bases, guanine and adenine, also separated as in Figure 4.6C. As well as the two related nucleosides, guanosine and adenosine have very good separation in Figure 4.6D. In all cases very well separated peaks were observed.

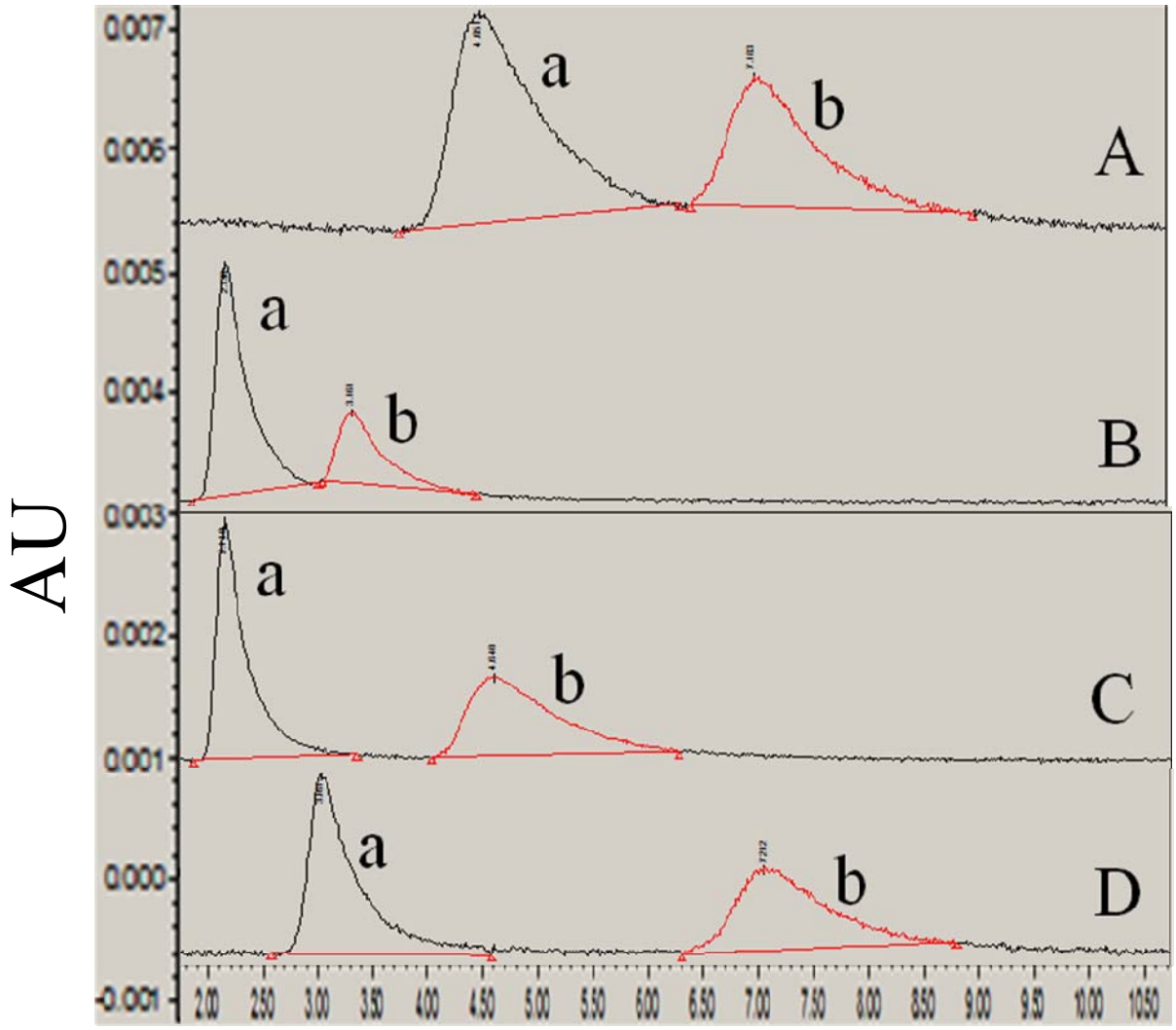


Figure 4.6. *HPLC chromatograms of a mixture of (A) adenine (a) and adenosine (b), (B) guanine (a) and guanosine (b), (C) guanine (a) and adenine (b), and (D) guanosine (a) and adenosine (b). (1.0 ppm each) in every mixture. Other conditions, as in Figure 4.1.*

4.5.2. Simultaneous Determination of Nucleic Acid Constituents

As mentioned above, many scientists have reported on the simultaneous determination of multiple bioanalytes. Keating and Natan used bar-coded microrods for the simultaneous analysis of multiple bioanalytes (103), Mirkin' s group reported on that scattering light from different sized particle tags could be used for optical assays of multiple targets (106).

In the present work we focus mainly on the development of electrochemical techniques for simultaneous analysis of nucleic acid constituents.

4.5.2.1. Using Electrochemical Methods

The simultaneous measurements were done using square wave voltammetry at glassy carbon paste electrode surfaces. Figure 4.7 shows the voltammogram of a mixture 1.0 ppm guanine, 1.0 ppm adenine and 5.0 ppm adenosine. Well defined peaks with very good resolution were obtained. Similar study was done for a mixture of 1.0 ppm guanine, 5.0 ppm guanosine and 5.0 ppm adenosine as can be seen in Figure 4.8.

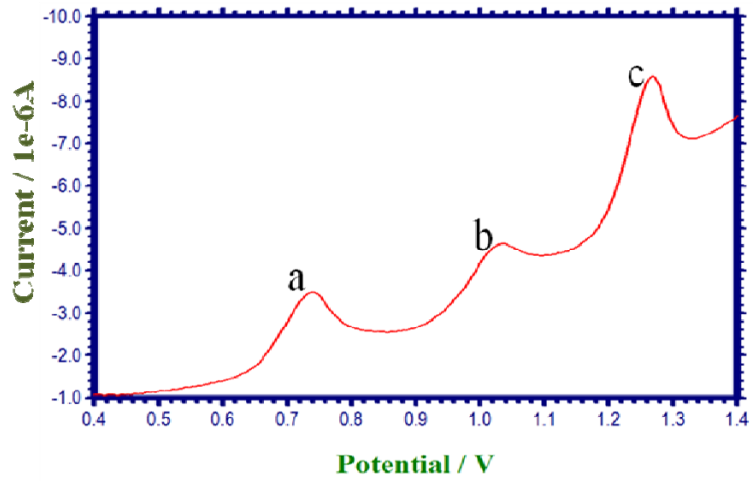


Figure 4.7. *Square-wave stripping voltammogram for a mixture of 1.0 ppm guanine (a), 1.0 ppm adenine (b), and 5.0 ppm adenosine (c) using GCPE. Other conditions, as in Figure 4.3.*

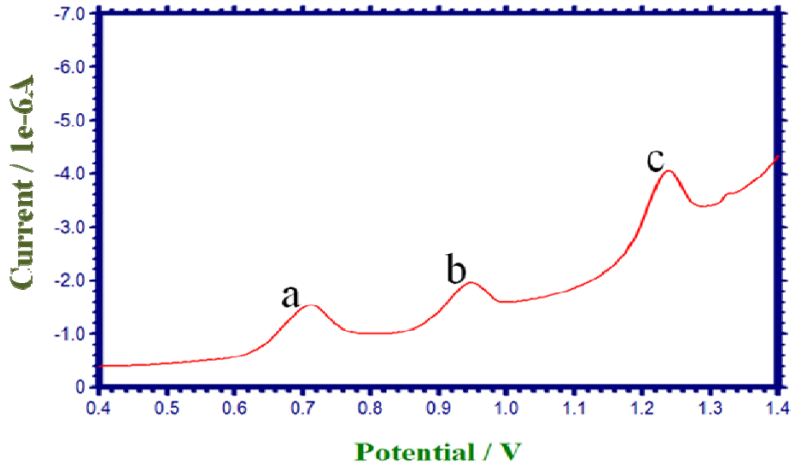


Figure 4.8. *Square-wave stripping voltammogram for a mixture of 1.0 ppm guanine (a), 5.0 ppm guanosine (b), and 5.0 ppm adenosine (c) using GCPE. Other conditions, as in Figure 4.3.*

4.5.2.2. Using High Performance Liquid Chromatography (HPLC) Method

The simultaneous measurements were successfully done using HPLC method. Figure 4.9 shows the chromatogram of a mixture of 1.0 ppm (guanine, adenine and adenosine). Well defined peaks with very good resolution were obtained. Same study was done for a mixture of 1.0 ppm each of (guanine, guanosine and adenosine) as can be seen in Figure 4.10.

Well separated peaks with good height appear for a mixture of the four analyte (guanine, guanosine, adenine and adenosine) in Figure 4.11.

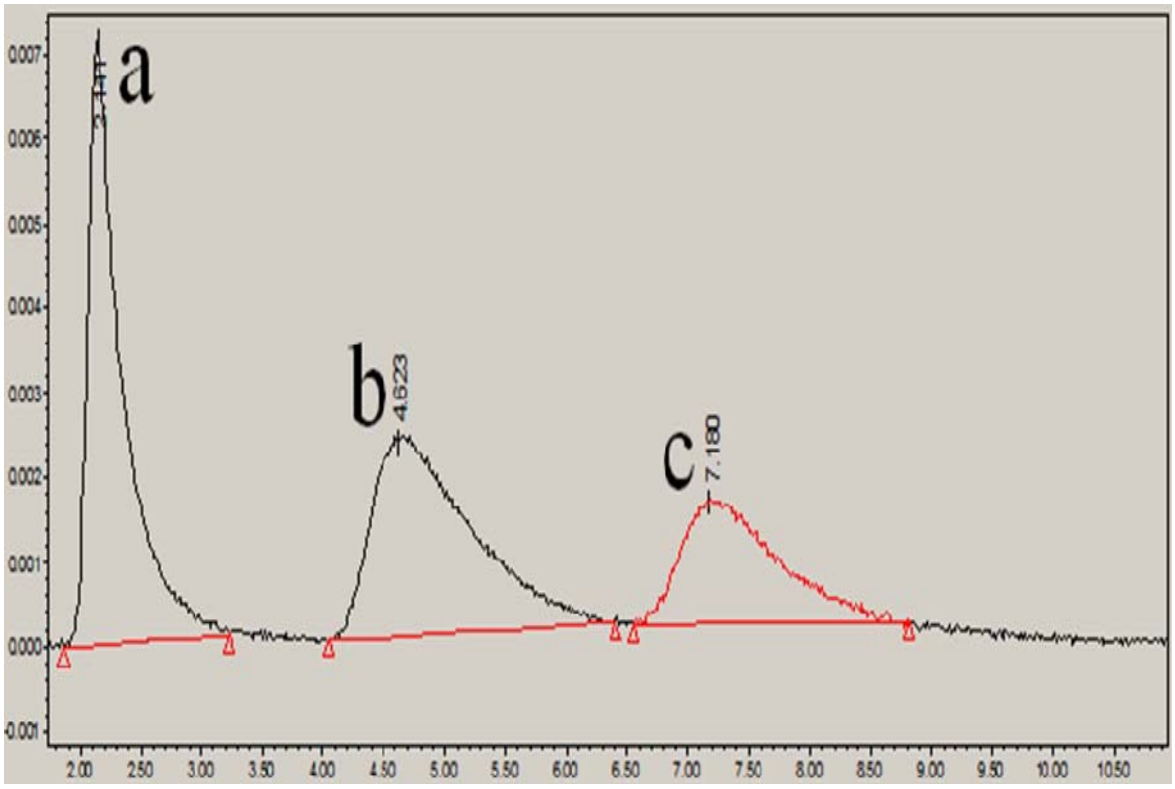


Figure 4.9. *HPLC chromatograms of a mixture of guanine (a), adenine (b) and adenosine(c) (1.0 ppm each). Other conditions, as in Figure 4.1.*

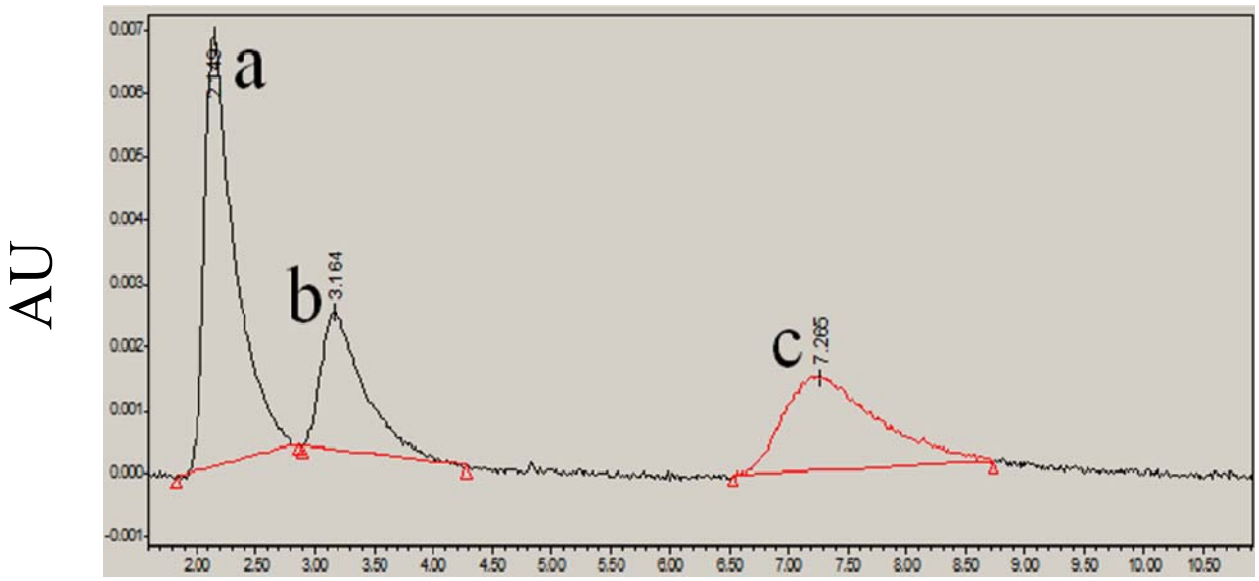


Figure 4.10. *HPLC chromatogram of a mixture of guanine (a), guanosine (b) and adenosine (c) (1.0 ppm each). Other conditions, as in Figure 4.1.*

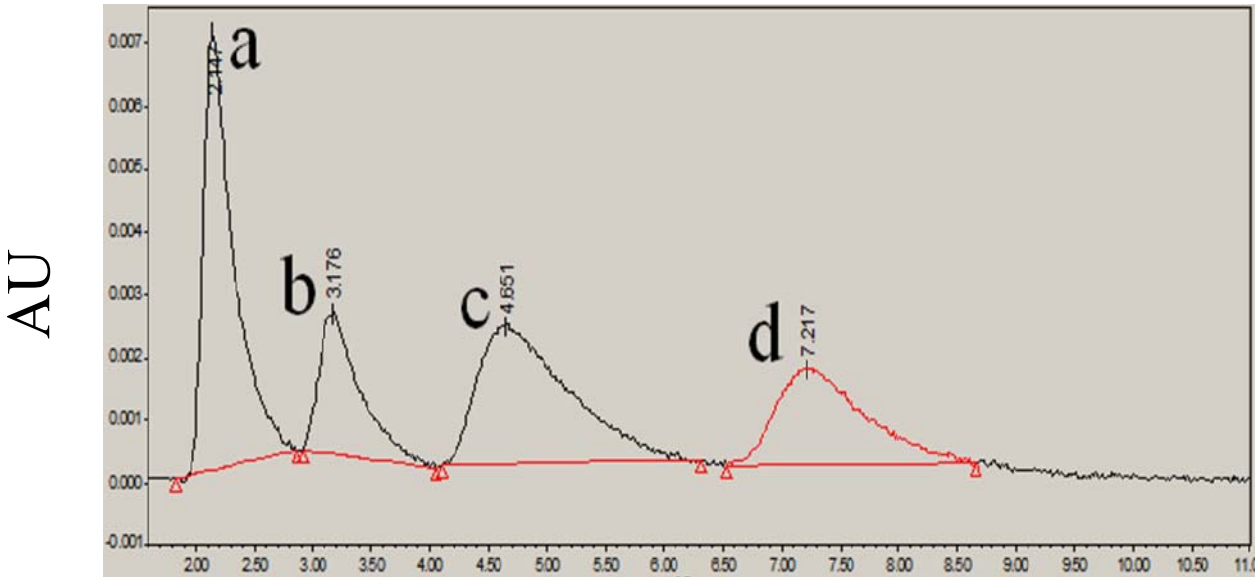


Figure 4.11. *HPLC chromatogram of a mixture of guanine (a), guanosine (b), adenine (c) and adenosine (d) (1.0 ppm each). Other conditions, as in Figure 4.1.*

4.5.3. Detection of Nucleic Acids

As an important application, the developed detection methods were tested for direct determination of ssDNA samples in absence and in presence of copper.

4.5.3.1. In Absence of Copper

Figure 4.12 shows the square wave voltammograms of acid-treated (digested) 10 ppm ssDNA solution at glassy carbon paste electrode surfaces. Figure 4.12 exhibits two oxidation peaks, at +0.91 V (guanine) and +1.23 V (adenine), following the acid treatment and a 1.0 min accumulation. A substantial enhancement at the same potential positions of the purines peaks is observed when adding different concentration from both of these purines.

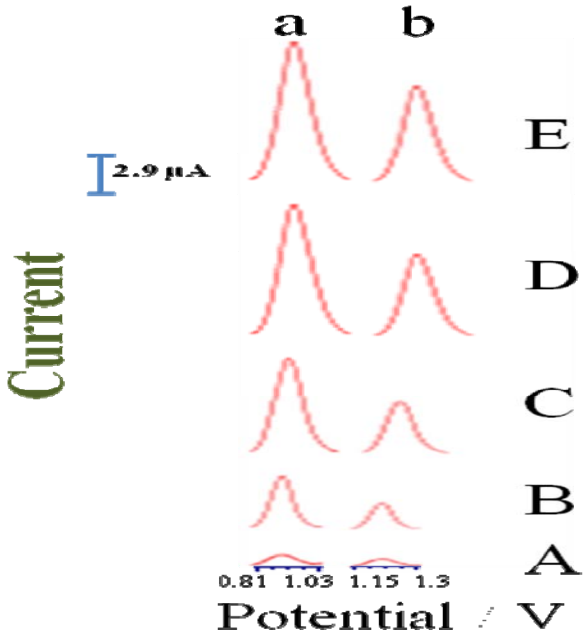


Figure 4.12. *Square-wave stripping voltammograms for undigested (A) and digested (B) 10 ppm ssDNA solution. Subsequent additions of a mixture of 1.0 (c), 3.0 (D), and 5.0 (E) ppm of guanine (a) and adenine (b) at GCPE. Phosphate buffer solution (0.2 M, pH 6.0). Electrode pretreatment, 1.0 min at +1.7 V; Accumulation time, 2.0 min at +0.5V.*

4.5.3.2. In Presence of Copper

Recent studies have demonstrated that the presence of copper ions enhances the electrochemical response of purine bases and leads to ultrasensitive DNA detection (32). Figure 4.13 displays square wave voltammogram signals for 10 ppm "acid-digested" ssDNA, in absence (B) and in presence (C) of copper. An enhancement of the guanine and adenine peaks upon adding copper was observed (C vs. B). Further increases in the height of both guanine (a) and adenine (b) peaks are observed with the addition of more concentration of these analytes (D, E and F)

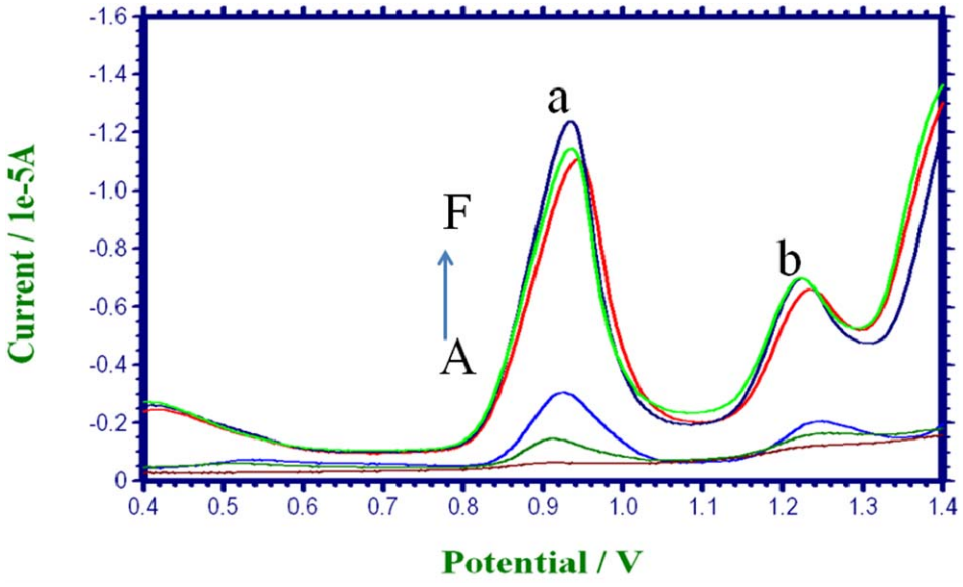


Figure 4.13. *Square-wave stripping voltammograms for 10 ppm ssDNA undigested (A) and digested in absence (B), and presence (C) of 2.0 ppm Cu(II). Subsequent additions of a mixture of guanine (a) and adenine (b) of 3.0 (D), 4.0 (E). and 5.0 (F) ppm each. Phosphate buffer solution (0.2 M, pH6.0). Electrode pretreatment, 1.0 min at +1.7 V; Accumulation time, 2.0 min at -0.05V.*

CHAPTER 5

5. Conclusion and References

5.1 Conclusion

In this work we have focused our attention mainly to the electroanalysis of adenine, guanine, adenosine and guanosine as important components of nucleic acid constituents using different carbon composite electrode materials. Also we have demonstrated for the first time the use of glassy carbon paste electrode for the electrochemical determination of these compounds. Our findings indicated that the glassy carbon paste electrode is the best among all materials have been tested. So it can be readily extended to other biological assays, particularly other nuclei acid constituents e.g. thymine, uracil, etc.

We have also found that different elements such as copper ions Cu(II) increase the signal of our analytes. Interference study has been done using different organic compounds, the obtained results indicated that the developed method has a good selectivity for detection of the analytes under investigation.

As application part we determined succesfully these compounds in biological sample (Human Urine), other important application has been done, which is detection of ssDNA in absence and presense of copper.

Finally, we have compared the developed electrochemical methods with another analytical technique which is HPLC. Table 5.1 summarizes the results which proved that electrochemical methods are faster, 3.0 min. vs. 10 min for HPLC, consume less solvent and require smaller amount of samples to detect.

Table 5.1 A comparison between electrochemical and HPLC detection methods

Technique	**Analysis Time**	**Solvent**	**Sample Volume**
Electrochemical	Max: 3min	1.0 ml	1.0 µl
HPLC	Min: 10min	15.0 ml	45.0 µl

5.2. References

1- C.M. A. Brett, *Pure Applied Chemistry*, 73, 1969, 2001

2- M. Grotheer, R. Alkire, and R. Varjian, *The Electrochemical Society Interface*, 52, 2006

3- G. K. Budnikov, *Journal of Analytical Chemistry*, 55, 1014, 2000

4- J. Wang, Analytical Electrochemistry, VCH Publishers, New York, 1994

5- A.J. Bard, Electrochemical Methods Fundamentals and Applications, 2nd Ed., John Wiley and Sons, Inc., New York, 2001

6- P.N. Bartlett, and J.M Cooper, *Journal of Electroanalytical Chemistry*, 362, 1, 1993

7- D.N. Gray, M.H. Keyes, and B, Watson, *Analytical Cemistry*, 49, 1067, 1977

8- S.J. Updike, and G.P. Hicks, *Nature*, 214, 986, 1967

9- Nucleosides, nucleotides and nucleic acids, Taylor and Francis Publisher, 2000

10- M. Mandelkern, J. Elias, D. Eden, and D. Crothers, *Journal of Molecular Biology*, 152, 153, 1981

11- IUPAC Commission on Nomenclature of Organic Chemistry (CNOC), *Journal of American Chemical Society*, 82, 5545, 1960

12- M. E. D. S. Frizzo, D.R. Lara, A. D. S. Prokopiuk, C. R. Vargas, C. Z. Salbego, M. Wajner, and D. O. Souza, *Cellular Molecular Neurobiology*, 22, 353, 2002

13- E. Palecek, *Nature*, 188, 656, 1960

14- V. Vetterl, *Journal of Electroanalytical Chemistry*, 19, 169, 1968

15- V. Vetterl, *Collection of Czechoslovak Chemical Communications*, 31, 2105, 1966

16- P. Singhal, and W.G. Kuhr, *Analytical Chemistry*, 69, 3552, 1997

17- E. Palecek, *Analytical Biochemistry*, 108, 129, 1980

18- E. Palecek, *Analytica Chemica Acta*, 174, 103, 1985

19- E. Palecek, and M. Fojta, *Analytical Chemistry*, 73, 74, 2001

20- H. Berg, and H. Bar, *Studia Biophysica*, 3, 133, 1967

21- H. Berg, and K.Z. Eckardt, *Nature*, 25, 362, 1970

22- E. Rojas, L.A. Herrera, L.A. Poirier, and P. Ostrosky-Wegman, *Mutation Research*, 443, 157, 1999

23- R. Cini, M.C. Burla, A. Nunzi, G.P. Polidori, and P.F. Zanizzi, *Journal of the Chemical Society, Dalton Transactions*, 2467, 1984.

24- S. Missailides, and J. Anastassopoulou, *Asian Journal of Physics*, 6, 481, 1997

25- M. Egli, *Chemistry & Biology*, 9, 277, 2002

26- A. Kawde, *International Journal of Nano and Biomaterials*, 2, 52, 2009

27- H. Xu, X. Mao, Q. Zeng, S. Wang, A. Kawde, and G. Liu, *Analytical Chemistry*, 81, 669, 2009

28- Z. Dai, A. Kawde, Y. Xiang, V. Bhavanandan, J. La Belle, L. Joshi, and J. Wang, *Journal of the American Chemical Society*, 128, 10018, 2006

29- T. Tangkuaram, J. Q. Gerlach, Y. Xiang, A. Kawde, Z. Dai, V. P. Bhavanandan, J. T. La Belle, W. Veerasai, L. Joshi, and J. Wang, *Analyst*, 131, 889, 2006

30- J. Hansen, J. Wang, A. Kawde, Y. Xiang, K. Gothelf, and G. Collins, *Journal of the American Chemical Society*, 128, 2228, 2006

31- N. Dolinnaya, M. Jan, A. Kawde, T. Oretskaya, V. Tashlitsky, and J. Wang, *Electroanalysis*, 18, 399, 2006

32- M. Rodriguez, A. Kawde, and J. Wang, *Chemical Communications*, 34, 4267, 2005

33- A. Kawde, M. Rodriguez, T. Lee, and J. Wang, *Electrochemistry Communications*, 7, 537, 2005

34- J. Wang, A. Kawde, and M. Jan, *Biosensors and Bioelectronics*, 20, 995, 2004

35- A. Kawde, and J. Wang, *Electroanalysis*, 16, 101, 2004

36- J. Wang, A. Kawde, and M. Musameh, *Analyst*, 128, 912, 2003

37- J. Wang, A. Kawde, M. Musameh, and G. Rivas, *Analyst*, 127, 1279, 2002

38- J. Wang, and A. Kawde, *Analyst*, 127, 383, 2002

39- J. Wang, and A. Kawde, *Electrochemistry Communications*, 4, 349, 2002

40- J. Wang, A. Kawde, A. Erdem, and M. Salazar, *Analyst*, 126, 2020, 2001

41- J. Wang, D. Xu, A. Kawde, and R. Polsky, *Analytical Chemistry*, 73, 5576, 2001

42- J. Wang, M. Jiang, and A. Kawde, *Electroanalysis*, 13, 537, 2001

43- J. Wang, and A. Kawde, *Analytica Chimica Acta*, 431, 219, 2001

44- J. Wang, U. Kirgoz, J. Mo, J. Lu, A. Kawde, and A. Muck, *Electrochemistry Communications*, 3, 203, 2001

45- J. Wang, A. Kawde, E. Sahlin, C. Parrado, and G. Rivas, *Electroanalysis*, 12, 917, 2000

46- J. Wang, M. Jiang, A. Kawde, and R. Polsky, *Langmuir*, 16, 9687, 2000

47- J. Wang, A. Kawde, and E. Sahlin, *Analyst*, 125, 5, 2000

48- M. Ahmed, M. Ibrahim, Y. Temerk, and A. Kawde, *Electrochimica Acta*, 41, 2883, 1996

49- M. Ahmed, M. Ibrahim, Y. Temerk, and A. Kawde, *Montashefte Fur Chemie*, 127, 609, 1996

50- M. Ibrahim, M. Ahmed, Y. Temerk, and A. Kawde, *Analytica Chimica Acta*, 328, 47, 1996

51- M. Ibrahim, M. Ahmed, A. Kawde, and Y. Temerk, *Analusis*, 24, 6, 1996

52- M. Ibrahim, Y. Temerk, M. Ahmed, and A. Kawde, *Analusis*, 24, 84, 1996

53- D. M. Whynot, R. A. Hartwick, and S. Bane, *Journal of Chromatography A*, 767, 231, 1997

54- R. Sheng, T. M. Cotton, and F. Ni, *Analytical Chemistry*, 63, 437, 1991

55- A. M. O. Brett, V. Diculescu, and J. A. P. Piedade, *Bioelectrochemistry,* 55, 61, 2002

56- T. Vojtylová, D. Dospivová, O. Třísková, I. Pilařová, P. Lubal, M. Farková, L. Trnková, and P. Táborský, *Chemical Papers*, 63, 731, 2009

57- J. H. Zhang, L. Belardinelli, K. A. Jacobson, D. H. Otero, and S. P. Baker, *Molecular Pharmacology*, 52, 491, 1997

58- D. Kloor, K. Yao, U. Delabar, and H. Osswald, *Clinical Chemistry*, 46, 537, 2000

59- W. Sun, Y. Duan, Y. Li, T. Zhan, and K. Jiao, *Electroanalysis*, 21, 2667, 2009

60- J. Yang, G. W. Xu, H. W. Kong, Y. F. Zheng, T. Pang, and Q. Yang, *Journal of Chromatography B*, 780, 27, 2002

61- T. Rasmuson, and G. R. Bjork, *Acta Oncologica*, 34, 61, 1991

62- J. Yang, G. W. Xu, H. W. Kong, Y. F. Zheng, T. Pang, and Q. Yang, *Journal of Chromatography B*, 780, 27, 2002

63- P. J. F. Harrisy, *Philosophical Magazine*, 84, 3159, 2004

64- H. Lin, D. K. Xu, and H. Y. Chen, *Journal of Chromatography A*, 760, 227, 1997

65- A.M. Bond, P.J. Mason, J. Schiewe, and V. Vicente-Beckett, *Analytica Chimica Acta*, 345, 67, 1997

66- C.M.V.B. Almeida, and B.F. Giannetti, *Electrochemistry Communications*, 4, 985, 2002

67- E. Palecek, *Collection of Czechoslovak Chemical Communications*, 25, 2283, 1960

68- X. Cai, G. Rivas, P.A.M. Farias, H. Shiraishi, J. Wang, and E. Palecek, *Electroanalysis*, 8, 753, 1996

69- V. Brabec, and J. Koudelka, *Bioelectrochemistry and Bioenerg*etics, 7, 793, 1980

70- H. Shiraishi, and R. Takahashi, *Bioelectrochemistry and Bioenerg*etics, 31, 203, 1993

71- M. Steichen, Y. Decrem, E. Godfroid, and C. Buess-Herman, *Biosensors & bioelectronics*, 22, 2237, 2007

72- L. Trnkova, R. Kizek, and O. Dracka, *Bioelectrochemistry*, 55, 131, 2002

73- S. P. Li, P. Li, T. T. X. Dong, and K. W. K. Tsim, *Electrophoresis*, 22, 144, 2001

74- Y. X. Gong, S. P. Li, P. Li, J. J. Liu, and Y. T. Wang, *Journal of Chromatography A*, 1055, 215, 2004

75- W. Jin, H. Wei, and X. Zhao, *Analytica Chitnica Acta*, 347, 269, 1997

76- T. Helboe, and S. H. Hansen, *Journal of Chromatography A*, 836, 315, 1999

77- G. Dryhurst, and P.J. Elving, *Journal of Electrochemical Society*, 115, 1014, 1968

78- G. Dryhurst, and G.F. Pace, *Journal of Electrochemical Society*, 117, 1259, 1970

79- G. Dryhurst, *Analytica Chemica Acta*, 57, 137, 1971

80- M. L. Hao, *Drug Stand. China*, 4, 340, 2003

81- L. L. Mi, S. W. Zhang, J. J. Sun, Z. H. Wang, and X. K. Hong, *Chin. Trad. Pat. Med*. 25, 402, 2003

82- W. G. Stillwell, H. X. Xu, J. A. Adkins, J. S. Wishnok, and S. R. Tannenbaum, *Chemical Research in Toxicology*, 2, 94, 1998

83- A. J. R. Teixeira, J. H. G. Ampt, G. V. deWerken, J. G.Westra, J. F. C. Stavenuiter, and A. P. J. M. de Jong, *Analytical Biochemistry*, 214, 474, 1993

84- S. K. Yan, G. A. Luo, Y. M. Wang, and Y. Y. Cheng, *Journal of Pharmaceutical and Biomedical Analysis*, 40, 889, 2006

85- S. P. Li, P. Li, C. M. Lai, Y. X. Gong, K. K. W. Kan, T. T. X. Dong, K. W. K. Tsim, and Y. T. Wang, *Journal of Chromatography A*, 1036, 239, 2004

86- L. S. Li, M. Liu, S. L. Da, and Y. Q. Feng, *Talanta*, 63, 433, 2004

87- A. A. Magana, K. Wrobel, Y. A. Caudillo, S. Zaina, G. Lund, and K. Wrobel, *Analytical Biochemistry*, 374, 378, 2008

88- D. Huang, Y. Zhang, and X. Chen, *Journal of Chromatography B*, 784, 101, 2003

89- C. Bolin, and F. C. Pelaez, *Journal of Chromatography B*, 856, 121, 2007

90- C. Tommaso, B. Giuliana, A. Salvatore, and M. Daniela, *Rapid Communications in Mass Spectrometry*, 23, 3465, 2009

91- Z. Jianzhi, S. Changhui, C. Bo, and Y. Shouzhuo, *China Journal of Chinese Materia Medica* , 35, 67, 2010

92- F. Q. Yang, J. Guan, and S. P. Li, *Talanta*, 73, 269, 2007

93- W. Furst, and S. Hallstrom, *Journal of Chromatography*, 578, 39, 1992

94- K. F. Childs, X. H. Ning, and S. F. Bolling, *Journal of Chromatography B*, 678, 181, 1996

95- S. Giannattasio, S. Gagliardi, M. Samaja, and E. Marra, *Brain Research Protocol*, 10, 168, 2003

96- A. Brink, U. Lutz, W. Volkel, and W. K. Lutz, *Journal of Chromatography B*, 830, 255, 2006

97- H. Fan, S. P. Li, J.J. Xiang, C. M. Lai, F. Q. Yang, J. L. Gao, and Y. T. Wang, *Analytica Chimica Acta*, 567, 218, 2006

98- J. W. Xie, L. F. Huang, W. Hu, Y. B. He, and K. P. Wong, *Molecules*, 15, 305, 2010

99- C. Fu, L. Song, and Y. Fang, *Analytica Chimica Acta*, 399, 259, 1999

100- H. Y. Cheung, C. W. Ng, and D. J. Hood, *Journal of Chromatography A*, 911, 119, 2001

101- F. Q. Yang, L. Ge, J. W. H. Yong, S. N. Tan, and S. P. Li, *Journal of Pharmaceutical and Biomedical Analysis*, 50, 307, 2009

102- M. Han, X. Gao, J. Su, and S. Nie, *Nature Biotechnology,* 19, 631, 2001

103- B. Reiss, L. He, I. Walton, R. Cromer, C. Keating, and M. Natan, *Science*, 294, 137, 2001

104- J.A. Ferguson, F.J. Steemers, and D.R. Walt, *Analytical Chemistry*, 72, 5618, 2000

105- S.A. Brazil, P. Kim, and W.G. Kuhr, *Analytical Chemistry*, 73, 4882, 2001

106- T.A. Taton, G. Lu, and C.A. Mirkin, *Journal of American Chemical Society*, 123, 5164, 2001

107- I. Yangs, P. Ropp, and H. Thorp, *Analytical Chemistry*, 74, 347, 2002

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